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AVIAN TUBERCLE BACILLI IN THE UDDER OF A GOAT—CHANGE OF S TO R FORMS

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IN 1927, I reported in this journal the finding of avian tubercle bacilli in the milk from one half of the udder of a goat which some months previously had been injected subcutaneously with culture of avian bacilli. Avian bacilli persisted in the udder and during many years were cultivated at intervals from the mammary secretion.

In 1931 an interim report was published giving an account of the findings down to March of that year. The experiment had then lasted five years and avian bacilli were still present in the udder and, on recovery in culture, were found to be unchanged in cultural characteristics and in virulence for the fowl.

The goat was subsequently kept under observation for the purpose of answering three questions: (1) For how long can avian bacilli colonise the udder of a goat? (2) If they persist for a very long time will they eventually destroy the secretory epithelium of the udder? (3) Does the avian bacillus retain its distinctive characteristics during long residence in the tissues of the goat?

It was possible to recover avian bacilli from the secretion of the right half of the udder—the left half did not become infected—down to May, 1935, that is to say, 9 years and 2 months after avian bacilli were injected subcutaneously. From that date all cultures were negative. During more than 8 years the avian strain showed no alteration in cultural characteristics. Towards the end of the ninth year the purulent secretion from the right half of the mamma produced on potato two kinds of colonies, one kind being smooth and soft, forming in salt solution homogenous suspensions, the other dry and coherent and yielding granular suspensions.

In this report I give a summary of the clinical history of the goat, an account of the experiments with the mammary secretion and a description of the bacteriological characteristics and serological reactions of the cultures recovered from the mammary fluid.

History of the Goat.

The nanny, when two to three years old and having had one kid, was inoculated subcutaneously on March 10th, 1926, with 100 mg. of a culture of avian tubercle bacilli derived from the tuberculous liver of a chicken. At the seat of injection on the left side of the neck there formed a very large lesion which sloughed. The resulting excavation gradually closed up and seven months later was represented by a slight thickening of the skin. The left prescapular gland attained the size of a hen's egg, underwent softening, and on February 20th, 1928, as it was threatening to ulcerate the skin, was incised and emptied of pus. The pus contained moderately numerous tubercle bacilli which, however, were apparently dead since they did not grow when planted on egg slopes and incubated for some months. Early in 1929 the right precrural gland was found to be enlarged and fluctuant. On March 14th, 1929, the gland was aspirated and thick pus was withdrawn. Egg tubes sown with the pus, which showed acid-fast bacilli microscopically, remained sterile. There were no further clinical signs of tuberculosis and the general health of the animal remained good until a few days before her death from old age.

The goat was "dry" when inoculated with the avian culture and the mammary cisterns contained only small amounts of watery slightly milky fluid. Samples of this fluid withdrawn from each half of the udder at intervals of 1, 2, 3, 7, 20 and 28 days after the inoculation of the goat were tested for avian tubercle bacilli and found to be free of these organisms. The method of testing was by direct cultivation and by making cultures from the inguinal glands of guinea pigs which had been inoculated subcutaneously with the mammary fluids. No further tests were made until October 7th, 1926, seven months after the inoculation of the goat. On that date thick milk drawn from the right side of the udder yielded on centrifugation an abundant deposit of slimy pus and showed on microscopical examination degenerate pus-cells and a moderate number of acid-fast bacilli. The organisms were cultivated and proved to be tubercle bacilli of the avian type. From that date until May, 1935, avian bacilli persisted in the right half of the udder of the goat and were from time to time recovered in pure culture from the mammary secretion. There were, however, wide fluctuations in the numbers of tubercle bacilli present in the udder at different times. The fluctuations followed a definite sequence and corresponded to the pregnancies, of which there were four. Just before each parturition the right mammary cistern contained a small amount of thick pus which was rich in avian bacilli. After parturition this infective

matter was removed by the kids with the colostrum milk and for several months subsequently tubercle bacilli were not recovered in culture from the small amounts (0·1 c.c. or less) of milk tested. After the weaning of the kids the contents of the right half of the udder gradually diminished in amount and changed in character from milk to pus-like fluid, becoming progressively richer in tubercle bacilli until the end of the gestation period.

The last pregnancy terminated on April 30th, 1931, and several matings in the following years, the last in December, 1935, were not fruitful. After the last pregnancy the changes in the character of the right mammary secretion were the same as after previous pregnancies. Avian tubercle bacilli which were present in relative abundance in the pus from the right mamma just before parturition were eliminated with the colostrum milk and were not recovered again from the milk until six months later, when two colonies were obtained from 0·15 c.c. At the seventh month 0·1 c.c. produced two colonies. At the ninth month a fortieth of a c.c. yielded 50 to 80 colonies. Thereafter the secretion, as on previous occasions, rapidly diminished in amount and changed in character from milk to pus, at the same time becoming progressively richer in tubercle bacilli until October, 1933. For about four months after this date, that is, until March, 1934, the bacilli per unit volume were apparently at their maximum and afterwards began gradually to decline in numbers until May, 1935, when the small amount of serous fluid then obtainable yielded (on potato) only three " rough " colonies of avian bacilli. All cultures, the last on November 11th, 1936, made since May, 1935, remained sterile. After November, 1936, no fluid at all was obtainable from this half of the mamma.

The cultural experiments made from time to time, the last occasion on November 11th, 1936, with the secretion from the left half of the udder were invariably negative.

During the period of the experiment the nanny gave birth to six kids (four pregnancies). One kid was still-born and one died through mishap soon after birth; they showed on autopsy no sign of tuberculosis. The remaining four kids were reared on the dam and when some months old were tested with avian tuberculin by the double intradermal method. The tests on the kids were all negative although the nanny reacted vigorously to the same tuberculin. Two of the kids were killed when 4 and 8 months old and were found on *post-mortem* examination to be free from any sign of tuberculosis and cultures from neck and mesenteric glands remained sterile. The two remaining kids were used in other experiments.

Although as will be seen from Table I the thick purulent fluid withdrawn from the right mamma at the end of each gestation period was rich in tubercle bacilli it must not be assumed that the kids ingested very large numbers of these organisms. The amount of infective matter in the mamma before lactation began was, in fact, very small—not more than a drachm—and this was removed

with the colostrum milk, as shown by the failure to cultivate avian bacilli during the first few months of lactation. One may conclude therefore that kids are not susceptible to small doses of avian bacilli taken naturally with the food.

The nanny was frequently tested intradermally with avian tuberculins prepared at the Institute of Animal Pathology, Cambridge, and always reacted to the first injection, a second injection only once being given. Because she reacted so well, the nanny was used on several occasions—by Mr. H. R. Allen—for testing the potency of the avian tuberculins which he had prepared. The last tests with avian tuberculins were on July 25th, 1936, one old and one new sample being used. A good moderate reaction was obtained with each sample at the first injection. These last reactions, it may be pointed out, were given nearly 15 months after it was found possible to recover avian bacilli in culture from the mamma.

The goat was also tested with human and bovine tuberculins on several occasions and with johnin once. It was always necessary to give a second injection which produced a slight definite reaction in each instance.

My thanks are due to Mr. R. E. Glover, F.R.C.V.S., and Mr. H. R. Allen, M.R.C.V.S., for doing the tests.

The goat began to show signs of failing health about the middle of the second week of March, 1937. On March 17th the animal was down and unable to get up; on the next day she was chloroformed.

Autopsy.—Left prescapular gland small and irregularly divided by fibrous connective tissue; the glandular tissue showed no sign of tuberculosis; attached to one pole there was a small cretaceous mass, probably the remainder of the local lesion. The terminal mesenteric gland contained two irregular calcareous nodules, one the size of a barley grain, the other that of a millet seed. The caudal lobes of the lungs contained irregular cretaceous masses; in the left there was a large arborescent mass and some small isolated nodules; in the right there were a few small masses, one pipe-stem-like as if based on a bronchus. A cretaceous mass from each region (local, glandular and pulmonary) was examined microscopically but no acid-fast bacilli or elements were found. The right side of the mamma was atrophied and the teat canal obliterated. On section nothing of a tuberculous nature was seen. The supramammary lymphatic glands were normal.

Histological examination of udder (by Dr. J. R. M. Innes). Normal inactive gland showing more than moderate fibrosis, a few calcareous deposits and cystic dilatation of main ducts which contain an abundant colloid.

Bacteriological Characteristics of the Avian Bacilli recovered from the Mamma.

The culture inoculated into the goat was typically avian in characteristics, that is to say, it produced on artificial media moist slimy

growths which readily formed homogeneous suspensions in salt solution and was virulent for fowls but not for guinea-pigs. The bacilli were recovered from the secretion of the right mamma on numerous occasions—nearly 50—and until February 8th, 1935, all the cultures appeared to be identical with the original culture. The media used for cultures were egg and glycerin egg, but in February, 1935, a little of the undiluted untreated pus was also sown upon a glycerinated potato medium. Numerous colonies were obtained on the egg media and on the potato. Those on the different egg tubes were at first superficially all alike and not apparently different from colonies obtained on these media at other times, but on potato the colonies were of two distinct kinds. One kind had a smooth, shiny surface, was soft to the touch and when rubbed up in salt solution readily formed a homogeneous suspension. The other kind had a rough, warty surface, was dry and coherent and formed in salt solution a granular suspension which soon deposited.

On March 13th, 1935, a few drops of purulent fluid were expressed from the right teat. The pus was diluted and an amount of the dilution equal to 1/400 of the pus was sown upon each of two egg tubes. (No potato tubes were sown because the rough colonies had not then been observed on the potato tube sown in February.) Numerous colonies appeared on the egg tubes and a random scraping produced moist slimy growths on glycerin agar. On one of the primary glycerin egg tubes after long incubation a convoluted rather dry looking colony was observed. This was picked off and sub-cultured and produced on glycerin agar a thick dry creamy-white wrinkled layer which formed a granular suspension in salt solution.

On May 1st, 1935, only a small drop of slightly milky fluid was obtainable from the right teat. This, after treatment with KOH for one hour, was sown upon three glycerinated potato tubes. Three colonies appeared on one tube and none on the other two. The colonies after six months were dry, heaped up, cream-coloured and warty, and of the size of hemp-seeds.

Virulence.—In my interim report on this experiment I gave the results of tests on eight fowls with cultures obtained from the milk at intervals of 2, 3, 4, 4½ and 5 years after the injection of the goat. All the cultures were fully virulent for the fowl. During this period milk or pus drawn from the right mamma at intervals of 2¼, 3¼ and 4¾ years proved avirulent for the guinea-pig.

The strain was again tested as to its virulence for the fowl after 7 and 8½ years residence in the body of the goat; the cultures, inoculated intramuscularly caused general tuberculosis in three fowls, which died in 63, 61 and 42 days respectively.

Milk from the right mamma drawn on March 10th, 1934, eight years from the beginning of the experiment was injected subcutaneously into a guinea-pig. The guinea-pig was killed 121 days later and showed a pea-sized inguinal gland with pus in its centre and no disease elsewhere. A culture was obtained from the pus and tested

on a guinea-pig by subcutaneous inoculation. This animal, killed 77 days later, showed nothing at the seat of injection and only slight enlargement of a right inguinal gland from which a culture of avian bacilli was obtained. Both these guinea-pig strains, it may be mentioned, resembled the original strain in cultural characteristics and absorbed the agglutinin from an avian serum.

Four fowls were injected with milk or pus from the goat's udder. The following table gives the results of these experiments.

No. of Fowl.	*Date of Sample.*	*Dose of Milk or Pus.*	*Method of Injection.*	*Duration of Expt. Days.*	*Result.*
103	21.9.28	7·0 c.c.	Intramusc.	K.300	No sign of T.
184	17.7.34	2 drops	Subcut.	(On 107th day no lesion locally; therefore reinoculated)	
	1.11.34	1 drop	,,	K.297 after 1st injection	No lesions found
191	8.2.35	0·22 c.c.	,,	K.92	,, ,,
198	21.5.35	Washing from right teat	Intramusc.	K.177	,, ,,

Fowls 184 and 191 were tested with tuberculin a few days before they were killed. Neither reacted. The completely negative results in all the fowls were surprising. Fowls 103, 184 and 191 certainly received some living bacilli, since the fluids withdrawn on those dates yielded cultures. It would seem from the results of these experiments that fowls are able to destroy small numbers of avian bacilli injected into their tissues and are therefore not suitable as test animals for detecting the presence in a material of small numbers of avian bacilli.

The next tests of virulence were made with single colony strains of the S and R forms. The results, which are summarised in Table II, show that both S and R strains were highly virulent for the fowl and rabbit and not virulent for the guinea-pig. The avian bacilli, therefore, after residing 9 years and 2 months in the body of the goat were unchanged in virulence for the fowl, rabbit and guinea-pig.

Two R strains, after about two years' cultivation on artificial media, were tested on the rabbit and were found to have retained their virulence.

Cultural Characters.—As previously stated, no change in the cultural characters of the bacilli was noted until February 8th, 1935, when rough as well as smooth colonies appeared on a primary glycerin potato tube. On egg tubes sown at the same time as the potato tube numerous colonies grew, but all these looked alike and a general scraping yielded moist subcultures. On March 13th, 1935, the numerous colonies on egg were undifferentiated except one which was raised, convoluted and rather dry. Subcultured, this colony produced on glycerin agar typical dry coherent layers.

On the glycerin egg primary cultures of February 8th and March 13th no differences between the colonies, which were very numerous and small, were noted at first. After the cultures had been in the incubator a long time they were examined minutely with a lens. The margins of all the colonies were flat and grey with fern-like surface-markings and in one tube were separated from each other by narrow interspaces, forming a mosaic. The centres were small prominences mostly less than 1 mm. in diameter and on magnification appeared to be of two kinds, one smooth, shiny, whitish or slightly cream-coloured, the other light buff with a matt surface. Subcultures of one or two of the matt centres were successful and produced dry coherent growths on glycerin agar.

On one of the primary glycerin egg tubes of March 13th growth had spread on to the wall of the tube as a delicate grey ground-glass layer, which on magnification closely resembled the delicate films produced on broth by very dysgonic bovine bacilli. A subculture of this film produced on glycerin agar a dry coherent growth.

Other subcultures from the primary glycerin egg tubes produced soft slimy growths on glycerin-agar.

The characteristics of the rough strains in subculture were as follows: On plain egg thin soft whitish layers only a little less readily emulsified than the S form. On glycerin egg thick creamy layers with irregular ridged nodular and in places wrinkled surfaces; the growth when scraped up generally cohered and could not be as readily respread or emulsified as the S form. The distinctive cultural characteristics of the two forms are most clearly brought out by the use of glycerin agar, glycerinated potato and glycerin broth, and of these glycerin agar is the best for the purpose on account of its transparency. On this medium the growths have varied from thin grey ground-glass, to thick cream-coloured, uniformly wrinkled coherent layers; sometimes the thinner layers developed subsequently secondary warty colonies or heaped up brownish warty masses. The differences between the S and R forms on glycerin agar are clearly seen in quite young cultures when these are viewed by transmitted light; the S layers are translucent, moist-looking and homogeneous with a glistening periphery; the R growths are more opaque, drier, with ground-glass surfaces. On glycerin potato thick dry, creamy, warty or convoluted crumbly layers were produced. On glycerin broth growth was on the surface as a dry coherent pellicle. The growths on these three media were often indistinguishable from those of the eugonic human type. Rough strains were found to have retained their distinctive cultural characters after passage through the guinea-pig (5), the rabbit (3) and the fowl (2).

When it was established that the rough strains were stable and retained their dry coherent characteristics on subculture and after passage through animals, those primary cultures still in existence which had been obtained from the milk prior to February, 1935, were carefully examined for evidence of the R form. The cultures

obtained on March 10th, 1934, and all previous cultures showed no sign of the presence in them of rough elements either among the primary colonies or on subculture. On the other hand, a primary glycerin egg culture sown with milk on September 5th, 1934, showed among numerous small whitish or creamy-white smooth colonies, a millimetre or less in diameter, about a dozen raised light brownish colonies, 2 to 3 mm. in diameter, with dry granular surfaces. A subculture from one of the latter colonies produced on glycerin agar luxuriant dry wrinkled layers.

Thus the first evidence of the separation of the R form was obtained 8½ years after the S culture was injected into the goat. The demonstration of the R form in primary cultures on egg and glycerin egg as well as on glycerinated potato shows that the "dissociation" was not induced by growth on a particular medium but that this had already occurred during residence of the bacilli in the udder of the goat.

Serological Reactions.—The two forms of the avian bacillus obtained from the goat have been tested for agglutinability and absorptive capacity against a serum prepared from a rabbit with the same strain of avian bacillus which was used to infect the goat. This strain has preserved intact its original S characteristics after subcultivation (over 100 subcultures at 37° C.) in my laboratory for upwards of 13 years. The rabbit was immunised by intravenous injection of cultures killed at 65° C. for half-an-hour. Two different samples of the serum were used and these agglutinated the homologous strain completely at 1 in 400 and 1 in 800 respectively. The agglutination tests were done in small test tubes, 75 × 11 to 12 mm., and to each tube were added 0·5 c.c. of the diluted serum and 0·5 c.c. of the suspension containing 1·0 mg. of the bacilli. The tubes were placed in an incubator, or a water-bath at 55° C. for four to five hours and then transferred to an incubator at 37° C. until next day, when the readings were made. Two S strains from the goat agglutinated in flocculent clumps in the 1 in 50 dilution in one hour and in all the tubes up to 1 in 800 within four hours; on the following day the clumps had settled, leaving the supernatant fluid clear. Two other typical avian strains tested at the same time agglutinated in flocculent clumps completely up to 1 in 1600.

The R suspensions (eight strains used) on the other hand showed very fine clumps in the lower dilutions, after four hours at 55° C., which could be seen distinctly only on magnification (× 8). After 24 hours there was complete deposition as a finely granular layer, the titre varying with the different strains from 1 in 200 to 1 in 400. When shaken up the small granular clumps were less easily dissociated than the larger flocculent clumps of the S form.

Agglutinin absorption tests were also done, mainly with the sample of serum whose titre was 1 in 400. All the S strains from the goat (six tests) and other typical avian strains, including the homo-

logous, absorbed the agglutinin completely from the serum. In six tests with strains from primary warty colonies on potato or glycerin egg there was also complete absorption. This may have been due to the presence in the cultures of some S elements, very small amounts of which were shown to be capable of removing the whole of the agglutinin from the serum. In 15 tests with eight R strains, the majority recovered from animals, there was no absorption of the specific agglutinin for the homologous S culture, although the amounts of culture used to absorb the serum were relatively very large—often 100 to 150 mg. in 1·5 c.c. of a 1 in 25 dilution of the serum.

This is the second occasion on which the R form of the avian tubercle bacillus has been obtained from a mammal. The first strain was isolated by F. Griffith from the mesenteric glands of a sheep which had been found on slaughter in an abattoir to be affected with a chronic form of general tuberculosis, apparently of alimentary origin. Several of the mesenteric glands were calcareo-caseous throughout, the spleen contained scattered gritty caseous miliary tubercles and there were miliary caseous tubercles in the lungs. The bacteriological characteristics of the strain were determined by me in Cambridge and reported in this journal in 1925. The culture grew on glycerin agar, potato and broth like a mammalian tubercle bacillus, but was avirulent for the guinea-pig and of high virulence for the rabbit and fowl. Two possible explanations of the aberrant characteristics of the culture were advanced.

1. The strain was originally typically avian, but during residence in the tissues of the sheep the bacilli became modified in cultural characters.

2. Avian tubercle bacilli in their natural hosts (fowls) do not all conform to one cultural type.

Of the two explanations I preferred the second because it seemed to me unlikely that so marked a change in cultural characters as a result of residence in the sheep's body could occur without some alteration of pathogenic properties. This now seems to have been a mistaken view since in the experiment on the goat the change from the S to the R form actually occurred without any appreciable alteration in virulence. In this connection it is interesting to mention that A. Eastwood and F. Griffith (1914) obtained from a pig a culture with the pathogenicity of the avian bacillus which culturally resembled the eugonic human tubercle bacillus.

Another strain of this type, the original source and characters of which, however, are unknown, has been examined by me. It was sent to me in 1925 by Dr. Roodhouse Gloyne and was one of a number of cultures which Dr. Gloyne had received from Monsieur Spahlinger in 1913. An account of the bacteriological characteristics and serological reactions of this strain will be published in a further paper on " rough " avian strains.

TABLE I

THE RESULTS OF THE CULTIVATION EXPERIMENTS WITH THE SECRETION OF THE RIGHT HALF OF THE UDDER ARE SUMMARISED IN THE FOLLOWING TABLE.

Date of Sowing Cultures.	*Character of the Secretion.*	*Amount of Milk tested.*	*Number of Avian Colonies obtained.*
Six occasions between March 11 and April 8, 1926	Watery	Small quantities for direct cultures and 10·0 c.c. for each guinea-pig	None direct or through the guinea-pig
October 7, and 8, and December 31, 1926	Purulent.	A small drop for each egg tube	Numerous
	January 29, 1927, *still-born kid.*		
February 21, 1927	Milk	One drop	A dozen or more
April 5 and June 21, 1927	Thick Milk	,,	About two dozen
September 1, 1927.	,,	,,	About four dozen
November 23, 1927 and March 15, 1928	,,	,,	Numerous
	March 15, 1928, *two kids born*		
March 16, 1928	Milk	0·1 c.c. on each of 2 tubes	Two
April 16 and June 1, 1928	,,	0·1 c.c. on each of 3 tubes	None
June 23, 1928	,,	16·0 c.c. on a G.P.	None from the guinea-pig
June 25, 1928	,,	40 c.c. on a G.P.	Guinea-pig healthy; avian colonies from the inguinal glands
August 13, 1928	,,	Two drops	One
September 8, 1928	,,	,,	Eight
September 20 and October 16	,,	,,	Eighteen to twenty-four
December 17, 1928	Thick milk	0·1 c.c.	More than 300
Five occasions from March 11, 1929 to March 10, 1930	Thick, semi-purulent	0·1 c.c.	Extremely numerous, uncountable
	March 15, 1930, *two kids born*		
June 2, 1930	Milk	0·1 c.c.	None
Every month from June 27, to October 16, 1930	,,	0·1 c.c. on each occasion	One, three, 12, 24 and 80 respectively
November 17, 1930	,,	0·025 c.c.	Innumerable

TABLE I—*continued*

Date of sowing Cultures.	*Character of the Secretion.*	*Amount of Milk tested.*	*Number of Avian Colonies obtained.*
December 15, 1930	„	0·0025 c.c.	Approx. 120. Also from ing. gls. G.P. killed 102 days
January 19, 1931	Thick pus	0·00025 c.c.	Ten to fifteen
March, 1931	„	0·0005 c.c.	Twenty-four
April 28, 1931	„	0·05 c.c.	Innumerable
	April 30, 1931, *one kid born*		
Every month from June 30 to September 22, 1931	Milk	0·05 c.c.	No colonies on any of the occasions
October 22, 1931	„ (Goat nearly dry)	0·05 c.c.	One colony on each of two tubes. None on another
November 27, 1931	„	0·05 c.c.	One colony on each of two tubes
January 27, 1932	Milky fluid	0·025 c.c.	50 to 80 colonies each tube
March 21, 1932	„	0·025 c.c.	Rather more numerous
June 7, 1932	Thick, purulent	0·025 c.c. 0·0125 c.c.	Numerous 60 on one tube
October 17, 1932 ; January, March and July, 1933	Thick, resembling conds. separated milk	0·0025 c.c.	From 24 to 30 per tube
October 23, 1933	„	0·0025 c.c.	More than 300 on one tube
January 1, 1934	„	0·0025 c.c.	Tubes thickly covered
March, September, November, 1934	Less thick	0·0025 c.c.	Moderately numerous, fewer on last than first occasion
February 8, 1935	Thin purulent	0·0025 c.c.	Average 160 on 2 egg tubes. Rough and smooth colonies on potato
March 13, 1935	Pus	0·0025 c.c.	About as many as in February
May 1, 1935	Milky fluid	Very small quantity undiluted	Three rough colonies on potato
June 20, 1935	Slightly turbid fluid	1 small drop	Two tubes sterile
July 26, 1935	„	„	Two tubes sterile

TABLE II
COMPARATIVE VIRULENCE TESTS WITH R AND S STRAINS.

GUINEA-PIGS.

(*a*) *Rough Strains.*

No. of Animal.	*Source of Culture.*	*Dose in mgm.*	*Method of inoculation.*	*Duration of life (days.)*	*Results.*
7047	R. colony prim. potato 1.5.35	10·0	Subcut.	K.28	Local ulcer, 1 cm. Caseo-pus in right, and two caseous tubercles in left inguinal gland. Cultures from ing. gl. = numerous colonies
7048	„	1 col.	„	K.29	Small local ulcer. One focus ing. gland each side. Cultures from ing. gl. = numerous colonies.
7089	G.P.7047	1·0	„	K.150	No loc. les. Purulent nodule in left and two foci in right inguinal gland. One xiphisternal gl. purulent. Portal gland an opaque focus. Cultures from each ing. gl. negative ; portal gland yielded one warty colony.
7076	G.P.7048	1·0	„	K.123	No loc. les. One focus in ing. gl. each side. Cultures from one gland = one warty rugose colony.
7077		1·0	„	K.105	No loc. les. Right ing. gl. purulent. Left ing. and one xiphisternal gland small but softened. Cultures from ing. gl. = scattered colonies on egg and glyc. egg ; granular cols. on glyc. potato.
			(*b*) *Smooth strains.*		
7066	Primary potato 8.2.35	1 Col.	Subcut.	K.21	Local lesion. Pus in ing. glands. Numerous cols. obtained from the pus.
7092	G.P.7066	1·0	„	K.150	No loc. les. Both ing. gls. softened. Cultures from pus = one smooth colony on each of 3 glycerin egg tubes.
7093		1·0	„	K.518	Local scar. Barley-grain-sized caseo-calcareous nodule in right ing. gl. Cultures from latter = 16 colonies on 3 tubes.

RABBITS.

			(a) Rough strains.		
4254	G.P.7048	0·1	Intrav.	D.19	Lungs enlarged reddish grey and unusually closely infiltrated with minute grey tubercles mainly aggregated, some opaqye centrally Pleural sacs filled with fluid. C seous patch in a tracheal gland. Liver and spleen much enlarged, speckled with small opaque tubercles.
4255	,,	0·01	,,	D.104	Chronic G.T., exhibiting distribution of lesions characteristic of chronic avian infections. Disease relatively slight in the organs. No glands but most of the joints were affected.
4276	G.P.7047	0·01	,,	D.33	G.M.T., avian type. T.b. extremely numerous in liver and spleen.
4277	,,	0·01	,,	D.25	G.M.T., less severe than in 4276.
4528	Rab. 4277 2 years' cultivation	0·01	,,	K.80	G.T. moderate severity. R form only recovered in culture.
4527	Fowl 209 nearly 2 years' cultivation	0·01	,,	D.25	Acute G.T. R form only recovered in culture.
			(b) Smooth strains.		
4278	G.P.7066	0·01	Intrav.	D.21	T. of Yersin type. T.b. extremely numerous in the organs.
4279	,,	0·01	,,	D.20	T. of Yersin type. T.b. less numerous than in 4278.
4526	Direct $3\frac{1}{4}$ years' cultivation	0·01	,,	D.30	Acute G.T., macroscopic foci in liver, spleen and lungs.

TABLE II—*continued.*

FOWLS.

(*a*) *Rough strains.*

No. of Animal.	*Source of Culture.*	*Dose in mgm.*	*Method of inoculation.*	*Duration of life (days).*	*Results.*
211	R. colony 8.2.35 subcult.	12·0	Intramusc.	D.67	Left pectoral muscle extensively replaced caseo-necrotic substance. Liver much enlarged closely beset with opaque miliary and submiliary tubercles. Spleen, moderate number caseating tubercles. Lungs, a few tubercles. Marrow, scattered tubercles. Neck glands, many tubercles.
209	G.P.4047	10·0	,,	D.39	Large local lesion. Liver and spleen enlarged and closely beset with submiliary tubercles. Marrow, moderate number miliary tubercles.
210	,,	10·0	,,	D.48	Large local infiltration. Liver (ruptured) and spleen greatly enlarged and closely beset with submiliary caseating tubercles.
206	,,	0·01	Intrav.	D.32	Liver enormously, spleen moderately enlarged, both organs filled with tubercles, the spleen on section resembling a fish-roe. Scattered tubercles in lungs, marrow and left ventricle muscle.
207	,,	10·0	Intramusc.	K.97	Moderate G.T. widely disseminated, the spleen being the most severely affected organ.
			(*b*) *Smooth strains.*		
208	G.P.7066	10·0	Intramusc.	D.30	Liver and spleen enlarged and peppered with tubercles.
214	,,	10·0	,,	D.91	Typical G.T., severe in liver and spleen.
215	,,	10·0	,,	D.83	Typical G.T., liver and spleen much enlarged and closely beset with tubercles. Numerous tubercles in marrow and on serous surface of gizzard. Small tubercles in neck glands.

Summary of the Results of the Goat Experiment.

1. A strain of avian tubercle bacilli, which had localised in the right half of the udder of a goat soon after the subcutaneous injection of a culture, persisted there for a period of about 9 years and was grown on successive occasions from the udder secretions.

2. During the residence of the strain in the udder there was a change from the S to the R form of the avian bacillus. The R form first appeared as an admixture in a culture made 8½ years after the injection of the goat and was the only form in the last positive culture obtained 8 months later.

3. The R form differed from the S form in cultural characters and in agglutinability and agglutinin absorption. No differences in virulence between the two forms were shown in experiments on fowls, rabbits and guinea-pigs.

4. The goat died 11 years after injection. The right half of the udder was atrophied and showed no sign of tuberculosis either macroscopically or microscopically.

THE PRESENT STATUS OF ANAESTHESIA*

By J. G. Wright.

During recent years the subject of anæsthesia in veterinary surgery has received great attention, and many advances have been made. Great advantage should accrue from a critical review of the present situation and this Congress is the most appropriate venue at which to give it consideration.

Satisfactory anæsthesia is essential from two important standpoints ; the first—the humanitarian, and the second—that of technical efficiency.

The subject is a complicated one, for the veterinarian is called upon to deal with a number of species which exhibit great variation in size, temperament, and anatomical and physiological development. Not only does the response of each species to the various anæsthetic agents vary, but in addition, there is marked individual variation within each particular species. Again, animals fear and resent restraint, thereby increasing not only the technical difficulties associated with administration, but also the dangers which are inseparable from the use of anæsthetics. When selecting the method to be employed, not only has the projected operation to be considered, but in addition the particular species, breed, and the temperament and health of the individual.

Broadly speaking anæsthetic agents are of two distinct types.

1. Substances which have a selective, transient paralytic action on sensory nerves and nerve endings. They are applied in aqueous or oily solution by topical application to mucous or abraded surfaces ; by intradermal, subdermal or submucous infiltration ; and by peripheral or spinal perineural injection. The anæsthesia thus produced is classified as local or regional. The alkaloid cocaine and its salts were the first to be employed, but more recently they have been largely replaced by synthetic substitutes of which procaine hydrochloride (novocain) is the most important.

2. Agents which have a depressant and ultimately paralytic action on the central nervous system producing a progressive loss of consciousness and voluntary motor function. When this loss is only partial the term narcosis is applied, and when complete that of general anæsthesia. In the main these agents fall into two distinct groups, volatile substances, typified by chloroform and ether, which are administered by inhalation ; and non-volatile agents of which chloral hydrate, morphine and the derivatives of barbituric acid are the most important, which may be administered by a number of routes, including into the stomach or rectum, and by subcutaneous, intravenous or intra-peritoneal injection.

* A paper contributed to the XIIIth International Veterinary Congress, Switzerland 1938.

I would urge that the terminology, when referring to the action of the cerebral depressants, should be regularised. One finds the term narcosis applied irrespective of its depth, and it becomes difficult thereby to correlate the results of the various workers. It is suggested that the term narcosis be applied when the loss of consciousness and voluntary motor function is incomplete, and that it be classified as light, medium or deep, according to its degree. When, however, consciousness is completely lost, the term general anæsthesia should be applied.

In view of the short space at my disposal it is impossible to discuss adequately every type of anæsthesia in all species, and I propose to confine my remarks to narcosis and general anæsthesia in the horse and dog. In these species narcosis is indicated for all, other than the simplest surgical interferences, whereas in cattle the temperament of the species allows of extensive interference being performed under local or regional anæsthesia alone.

The Horse.

The horse is the subject which presents the greatest anæsthetic problems. Consequent upon the animal's size and weight, strength and agility, methods which are ideal in the other species have to be discarded. Needless to say the simpler and more rapidly performed the operation is, the easier it is to induce satisfactory anæsthesia, but for the more severe interferences it is seldom that perfectly satisfactory results are obtained by any single method, but a combination of two or even three may be found necessary.

Inhalation Anæsthesia.—Chloroform by inhalation was the first general anæsthetic to be used, and there are many experienced veterinary surgeons who consider that it is still the most effective method at our disposal. Generally the animal is cast and restrained before administration, although a method by which the drug is given in the standing position has long been in vogue in Great Britain. Whichever method is adopted the chloroform is generally administered by means of a cylindrical mask of canvas and/or leather, and an initial dose of 15 to 60 c.cm., applied on a sponge. Numerous modifications of the simple inhaler have been introduced fitted with inspiratory and expiratory valves. It has been the experience of most that it is necessary, to induce anæsthesia in a reasonable time, in all but very small subjects, to restrict the air supply, by closing the front of the mask. Undoubtedly partial asphyxiation occurs, and yet for rapidly performed interferences the method has given very good results. When, however, even and continuous anæsthesia for a period of 30 minutes or longer is required, the method is unsatisfactory. Not only do marked variations in the depth of anæsthesia occur, but there is a risk of death from respiratory failure. In addition post-anæsthetic lung complications may occur. Henkels[1] has devised an apparatus whereby, using a sealed mask, the horse inhales oxygen drawn through chloroform vapour, the latter being contained in a metal canister.

This would appear to be similar to the apparatus used for small animals, and whilst it is a definite advance upon the simple mask method, it is only applicable for use in institutions and not under farm conditions.

Assessment of the depth of anæsthesia present is difficult in this species. Whilst complete relaxation of the tail is good evidence of anæsthesia, it is not an exact guide as to its depth. The corneal reflex which is such a good guide in the small species is generally maintained in the horse almost to the point of bulbar paralysis. A striking feature in many horses when employing chloroform and allowing air is the entire absence of the so-called second stage of anæsthesia —that of narcotic excitement. A little initial, voluntary struggling is followed by a state of hypnosis during which the animal lies perfectly still. The state is often mistaken for anæsthesia, but the application of the knife quickly proves the contrary. The writer often takes advantage of this hypnotic state to facilitate operation under local or regional anæsthesia. Preliminary narcotisation is a most valuable adjunct to inhalation anæsthesia in all species. Not only is the degree of struggling and the risk of accident associated with casting greatly reduced, but, in addition, the whole course of anæsthetisation is much more regular. Quinlan, de Koch and Marais[2] in their work on splenectomy, probably the most formidable interference ever successfully performed in the species, adopted this method. The administration of medium narcotic doses of chloral hydrate by intravenous injection was followed by the inhalation of chloroform using an open mask.

A great advantage possessed by the volatile anæsthetics over the non-volatile, is the rapidity with which they are excreted once administration ceases. Even after deep chloroformisation a horse will be able to rise and stand in a period of 30 minutes or so. This is a matter of great importance, for the animal must be carefully attended until it has regained its feet.

Non-volatile Agents.—Chloral hydrate is one of the oldest and is still the most often employed non-volatile narcotic agent for the horse, and when every aspect of the subject is considered it is the best at our command, although it must be stated at the outset that it is far from being ideal. It is used to induce depths of narcosis varying from a light stupor to full general anæsthesia. It is administered by a variety of routes, including drenching, direct introduction into the stomach by the stomach-tube, by enema, and by intraperitoneal and intravenous injection. The difficulties associated with drenching require no reiteration. The stomach-tube method is much to be preferred and can be satisfactorily carried out in most working animals. It is not practicable in young, untamed subjects, whilst the nasal hæmorrhage it sometimes provokes may preclude the subsequent adoption of inhalation anæsthesia. Silbersiepe[3] adopts the method of withholding drinking water until the animal will drink chloral hydrate in water voluntarily. This method is impracticable in th

writer's clinic where it is the practice to operate not later than 24 hours after admission ; in fact, in a large number of cases, operation is performed quite soon after presentation. The intravenous method has many objections when employed in the standing position. The first is the bulk of the injection. Consequent upon the irritant nature of the drug it is essential that it shall be well diluted, and concentrations greater than 10 per cent. should not be employed. The injection, by gravitation, will take five minutes or more to complete, and it is difficult to maintain perfect stillness in a vigorous subject for such a long period. Not infrequently, movement results in the needle leaving the vein before satisfactory narcosis is attained. In addition, it gives rise to a risk of peri-venous abscess formation, although, in the author's experience, provided the gravitation method is employed, this risk is small. At the same time it is probably too great to be taken by practitioners dealing with valuable animals. The method is impracticable in young, untamed horses. The question arises, what dose should be given in the standing position ? This will depend upon the route of administration. If doses capable of inducing deep narcosis are administered intravenously, the onset will be rapid, but the animal may stagger and flounder for a minute or so before relaxing in the recumbent position. When working in the open this may not be of great consequence. In the writer's opinion, if the intravenous method is adopted when working in a confined space, light narcotic doses only should be employed. By this is meant that a state of sleepiness supervenes but the animal is still able to stand. This dose, in a healthy animal which has been fasted for not longer than 24 hours, is put at 2·5 to 3 grammes per 50 kgm. bodyweight.* When administration is by a route from which absorption is slower, *e.g.*, the stomach, this dose may, with advantage, be increased with the view to inducing medium or even deep narcosis—from 5 to 6 grammes. Provided that the stomach is empty results are fairly regular. The onset of narcosis is quiet and it attains its maximum depth in about 20 minutes. The animal should be cast about 10 minutes after administration. In the writer's opinion this method represents the great value of chloral hydrate in equine surgery. The animal lies quietly in the cast position ; the induction of full anæsthesia by the administration of chloroform by open methods is greatly facilitated, as also is interference under local or regional anæsthesia. It must be understood, however, that with the chloral hydrate alone, the animal is by no means anæsthetised ; in fact skin sensation is but slightly reduced, and anæsthesia must be completed by some other method.

If chloral hydrate is to be employed in full anæsthetic doses the intravenous route is the only one to be considered, and the animal

* Throughout this paper doses for the horse are given in grammes per 50 kilogrammes bodyweight, for the sake of regularity. Some authors give doses in mgm. per kgm., others in cgm. per kgm., and others still in gm. per 100 kgm. 50 kgm. is adopted as it approximates 100 lbs., a convenient unit of weight in countries which adopt the avoirdupois system.

should be cast and effectively restrained before administration to ensure that the whole quantity is slowly given. Marcenac and Lemetayer[4] found that 5·5 grammes gave consistently good results. Administration was carried out in the cast position using a 20 per cent. solution with sodium citrate, the injection occupying six to eight minutes. Dumas[5] confirms these findings and in addition shows that the safety margin is large, putting the fatal dose at 18·5 grammes. Despite this finding the writer is of the opinion that when full anæsthesia is induced, using a comparatively slowly excreted non-volatile agent, there is always a risk of respiratory failure occurring, and that this risk becomes greater in the horse because of the great difficulty in assessing the degree of anæsthesia present. A matter which has not received attention and which merits investigation is the action of the drug on the liver. The fact that the animal appears to make a complete recovery is not evidence that its organs have sustained no injury. Investigations into the action of similar halogen-containing compounds such as carbon tetrachloride have shown the opposite to be the case.

Another very important aspect to be considered is the duration of narcosis. When medium narcosis is induced, the animal will be able to rise and stand in from 45 to 60 minutes after administration. When deep narcosis is induced the period of recovery will be delayed for about two hours; whilst if general anæsthesia is attained it is improbable that the animal will be able to rise before three to four hours have elapsed. This is a matter of serious consequence for it is necessary that the animal shall be carefully watched and possibly restrained, for the period of recovery is sometimes attended by excitation; otherwise there is a risk that it will flounder about and possibly sustain injury. It is for this reason chiefly that the writer is of the opinion that chloral hydrate should be employed only in doses capable of producing narcosis of medium depth.

The recent introduction of a large number of synthetic narcotic agents has led to a trial of many of them in the horse. Avertin (tribrom ethyl alcohol) has become popular in human surgery, administration being by colonic infusion. In the horse this method is impracticable. The writer has found that, injected intravenously in the usual 3 per cent. solution, a dosage of 1·5 grammes induced transient general anæsthesia of from five to eight minutes duration, after which recovery was extremely rapid. Whilst such anæsthesia is too transient for any other than the simplest interferences, the use of avertin might with advantage be further investigated, the rapidity of recovery being an important factor.

Nembutal (sodium ethyl methyl-butyl barbiturate) has attained great prominence as a general anæsthetic in small animals, and its possible use in the horse has been investigated. The writer used it for the induction of general anæsthesia by two methods, *viz* :—

1. In the standing position, by the rapid injection of computed doses into the jugular vein;

2. In the cast and restrained position, by slow intravenous injection by gravitation, estimating the degree of narcosis as injection proceeded, and occupying five to eight minutes to induce full anæsthesia.

It was found that, in vigorous healthy subjects, a dose of 1 gramme in 6 per cent. solution provoked deep narcosis but analgesia was not complete, whilst a dosage of 1·4 grammes gave complete general anæsthesia. As was the case with chloral hydrate the duration of narcosis was a great disadvantage, for after general anæsthetic doses the animal was unable to rise for four to five hours. In several cases the period of recovery was accompanied by marked narcotic excitement. If the drug is administered in the standing position the dangers associated with floundering are encountered. Sweebe[6] states that a dose of 1·7 to 2 grammes (total dose) given intravenously will put a horse " out " on its feet and thus greatly facilitate operation under local anæsthesia. Marcenac, Carnus and Comby[7] have used ethobutyl-ethyl barbituric acid, and report that 1 gramme in 10 per cent. solution, by slow intravenous injection, will provoke satisfactory deep narcosis of 50 to 70 minutes duration, the animal being able to rise in from 90 to 105 minutes. The writer has been using a very similar agent—sodium soneryl, and the method being investigated at the moment is the administration of light narcotic doses in the standing position, and subsequent deepening by further injection after casting.

Of the use of the very short-acting barbiturates, Velu and Zottner[8] have reported on evipan sodium. They found that a dose of 1 gramme gives 25 minutes perfect anæsthesia. The writer's experience of this agent in the dog was that there was a marked tendency to frenzy during the period of recovery and for that reason he has refrained from using it in the horse. As yet we have no reports on the use of pentothal sodium.

Although the work which has been done on the application of these new agents in the horse has been of great value in the search for the ideal narcotic for this species, the greater part of it is of academic interest only, for the great cost of these agents excludes them from practical adoption, especially in farm animals.

Whilst most workers have been interesting themselves in the new synthetic agents, Amadon and Craig[9] have been investigating the soporific and analgesic properties of the vegetable alkaloid bulbocapnine. They find that 0·05 gramme injected subcutaneously produces a state of somnolence in the standing position with some reduction in response to external stimuli. Combined with morphine as an analgesic (0·01 gramme) it has been used as an adjunct to operation under procaine infiltration. Results, however, were variable and whilst some animals remained somnolent throughout, in others interference provoked marked, although transient, excitation.

The Dog.

This is the species in which the veterinarian has the greatest surgical scope, and during recent years, consequent upon the introduction of so many new narcotic agents, the whole subject of narcosis

and general anæsthesia has become revolutionised. In the writer's opinion nembutal and pentothal are of outstanding value.

Nembutal.—Nembutal anæsthesia is indicated for all extensive surgical interferences, in fact once exprience is acquired in the various methods of administration and confidence in the use of the agent established, veterinary surgeons will be inclined to adopt it for many minor interferences which were previously performed either under morphine narcosis with local or regional anæsthesia, or local anæsthesia alone, for an animal in a completely unconscious and relaxed state greatly facilitates the performance of efficient surgery.

The writer is convinced, after a very considerable experience in the use of the drug, that when properly administered it is almost without risk. It was first employed in America in 1931, and B. H. Kreutzer[10] was the first to record its use. At the outset it was being administered orally or by intra-peritoneal injection and despite variations in results it became quite evident that it represented a marked advance on the older methods of inhalation anæsthesia. These methods necessitated the computation of an anæsthetic dose, and this was originally put at 1/5th of a grain per lb. bodyweight (0·028 gramme per kg.)* for healthy animals. Results were more regular when the intra-peritoneal rather than the oral route of administration was adopted. In the latter case the presence of food in the stomach caused great variation. At the same time using this dosage by intra-peritoneal injection did not give entirely consistent results, for cases were met in which narcosis only (often accompanied by excitation) was obtained, whilst in others anæsthesia became alarmingly deep, and in a very occasional case fatal. It soon became evident that whilst for healthy animals of from 25 to 35 lb. (11 to 15 kg.), this dosage gave satisfactory results, in the case of animals below these weights the dose must be increased, and for those above them reduced. Wright[11] recommended the following :—

Bodyweight		Dose	
lbs.	kg.	grains	grammes
10	4·5	2·5	0·16
15	7·0	3·5	0·22
20	9·0	4·5	0·28
25	11·0	5·25	0·33
30	13·5	6·0	0·37
35	16·0	6·5	0·4
40	18·0	7·0	0·44
50	22·5	8·25	0·52
60	27·2	9·0	0·56

Using this dosage the drug was employed by the writer and his colleagues in 300 reasonably healthy subjects. Results were generally consistent, although there was variation in the depth of anæsthesia obtained. In two it proved fatal. At the same time from the standpoint of toxicity the drug compared more than favourably with the

* When referring to doses by bodyweight in the dog, figures will represent grains per lb. in the avoirdupois system, and grammes per kilogramme in the metric.

inhalation anæsthetics. It was soon recognised that the adoption of computed doses was not the ideal method of using non-volatile anæsthetic agents, for quite apart from bodyweight there are many factors which influence their action even in healthy animals, whilst when dealing with diseased subjects variations must be marked. As the result of liver, cardiac or renal dysfunction susceptibility becomes increased, and as there is no accurate clinical method of assessing this increased susceptibility accidents are inevitable. At this point it was decided to employ the method of intravenous injection, but instead of giving an estimated dose, to make the injection slowly and to assess the degree of narcosis as injection proceeded, stopping when the required degree was obtained. By this means varying depths of anæsthesia can be induced at will ; overdosage can be avoided, and the safety factor is greatly increased. In the writer's clinic the method has been employed in over 1,500 cases, and in more than 600 consecutive cases without a death attributable to the anæsthetic.

The time of onset of narcosis will vary according to the route of administration. When the intravenous method is employed it will have attained its maximum depth at about the end of the fourth minute. As a general rule there is no period of narcotic excitement, but this is not invariable. If narcosis only is induced the animal may become hyperæsthetic and stimulation may provoke uncontrollable movement and/or barking. Thus the aim should always be to produce anæsthesia, its depth depending upon the interference to be performed. It remains at its original depth for a period of a half to one hour after which it progressively lightens. The duration of narcosis also tends to vary according to the route of administration, and it is least prolonged after intravenous injection when it is a matter of four hours or so. During the period of recovery there is a tendency in some individuals to narcotic excitement, and thus the recovering animal should be kept perfectly quiet. If an overdose is given, paralysis of the respiratory centre will occur. In such cases death generally occurs in a period of 10 to 15 minutes after induction. It is most likely to occur in very small subjects in which the total dose is small and there is a tendency to make the injection too quickly. Delayed poisoning does not occur as the healthy liver is capable of rapidly and completely breaking down the drug. It is when there is some interference with liver function that especial care must be taken. Prolonged fasting by reducing the glycogen reserves increases susceptibility, as also do degenerative conditions of the organ associated with prolonged toxæmia, neoplasm disease, etc. At the same time the drug does not appear to have a toxic action on the myocardium, for it has been used on many aged dogs with extensive valvular disease without ill-effect. Again it has been found that chronic nephritis does not increase susceptibility provided that the animal is not in the acute uræmic state at the time. In all cases in which increased susceptibility is suspected the risk of poisoning may be greatly reduced and the duration of narcosis lessened by the administration of glucose-saline solution, preferably by intravenous injection.

Administration and Assessment.—Injection is made slowly, the object of the method being to induce general anæsthesia progressively, in a period of three to four minutes, and until experience is gained it is well to time the injection. In healthy dogs it is the writer's practice to inject about two-thirds of the approximate dose in a period of one minute, and then wait for a further minute while the drug is being absorbed. From this point additional injections of small quantities are made with a pause of 20 seconds or so after each. The degree of narcosis is being assessed as the injection proceeds. In acutely toxic cases injection must be made very slowly from the commencement, for, not infrequently, in such conditions as pyometra, prolonged dystocia, etc., it is found that half the normal dose will provoke deep anæsthesia. The first indication is the onset of muscular relaxation and this is demonstrated by the head when lifted falling limply. If the mouth is opened at this point it will be found to provoke movement of the tongue. This will vary, according to the depth present, from a complete yawn to a slight curling of the tip. A little more of the solution is injected and after an appropriate wait the jaws are again opened. One's object is to reach the point at which the jaws are completely relaxed, and the tongue hangs limply. When this is attained light anæsthesia is present. This degree is suitable for all superficial operations. It may be decided to deepen anæsthesia by the inhalation of ether, or by administering more nembutal. If the latter is selected, the pedal reflex is used as the index of depth, slowly continue the injection until the response to vigorous pinching of the web between the digits is simply a slight contraction of the limb muscles without appreciable movement. By this time anaesthesia is becoming deep and beyond this point one should not proceed.

Morphine-Nembutal.—There is a summation of the actions of morphine and nembutal in the dog. The combination has advantages over nembutal alone in certain cases ; in subjects which it is difficult to restrain effectively without preliminary morphine narcotisation, and in very large dogs, in which the dose of nembutal alone would be large and costly. In the writer's experience the optimum narcotic dose of a morphine salt is one-thirtieth (0·005) for animals up to 60 lbs. (27 kg.), whilst for those over this weight it may be slightly reduced. Giving this dose by subcutaneous injection it has been found that if nembutal is administered by slow intravenous injection three-quarters of an hour later, the dose of the latter becomes reduced to from two-thirds to one-half of the normal dose. With the combination the duration of anæsthesia is shortened and there is generally evidence of lightening in 30 minutes ; the total period of light narcosis, however, is prolonged due to the morphine. Morphine has many disadvantages which there is not space to discuss in this paper, and speaking generally the writer much prefers the use of nembutal alone to that of the combination.

Pentothal (sodium ethyl methyl-butyl thio-barbiturate).—The duration of nembutal narcosis may be considered too long for the many simple and rapidly executed interferences we are called upon

to perform, in which full anæsthesia for a period of 10 to 15 minutes is quite adequate. The recently introduced pentothal sodium, one of the group of very short-acting barbiturates, is most valuable in this connection. Its use in canine surgery was first recorded by Sweebe[12], and it is administered by slow, intravenous injection in a manner similar to that described for nembutal. Its anæsthetic potency is similar to that of nembutal, but there would appear to be several factors which influence both the dose required to induce anæsthesia and its duration. The most important of these is the rate at which injection is made. When given rapidly, that is in from $\frac{1}{2}$ to 1 minute, the onset of anæsthesia is abrupt and there may be considerable shock with a possibility of threatened respiratory failure. Whilst the dose required is small (one-eighth to one-seventh—0·016 to 0·02), the duration of anæsthesia is very short—from three to five minutes. When the drug is administered more slowly and from three to five minutes is occupied during induction, the dose taken is larger (one-sixth to one-fourth—0·023 to 0·03) whilst the duration of anæsthesia is longer—from 10 to 20 minutes. It is advised that in all cases slow injection shall be adopted, continuing until the jaws are completely relaxed, and the tongue hangs limply out of the mouth. Using this method the writer has had most satisfactory results and no death has occurred. The type of anæsthesia resembles that obtained with nembutal. The first indication that anæsthesia is passing off is that the jaws stiffen and the tongue curls when the mouth is interfered with. There may be licking of the nose. From this point recovery is rapid, and the animal is obviously quite conscious in a period of 30 to 45 minutes after induction. Limb co-ordination, especially that of the hind limbs, is delayed and the animal will stagger in a drunken fashion, for an hour or so. There is no post-anæsthetic malaise or vomiting, nor has there been any clinical indication of injury to the liver or kidneys. In addition there is no tendency to excitation during the period of recovery. This is in marked contrast to the action of evipan sodium. In America the combination morphine-pentothal has been employed with the object of increasing the duration of anæsthesia for three-quarters to one hour. The writer does not favour this combination because of the variations in individual response to morphine. When longer anæsthesia is required it is recommended that nembutal and not pentothal is employed.

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SEASONAL HYPOMAGNESAEMIA OF THE BOVINE, WITHOUT CLINICAL SYMPTOMS

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Introduction.

Over the past five years a considerable volume of miscellaneous data on the variation in level of serum magnesium in metabolic disorders of the larger domesticated animals has accumulated in this laboratory. In attempting to sort out data for publication and correlate clinical symptoms with conditions of occurrence, obscure annual and seasonal variations were encountered but since an unexpected type of seasonal variation, without clinical symptoms at all, was observed on an unmistakable scale during the year 1937-1938 it was considered best to clear the ground by discussing this first.

The general position in regard to the association of hypomagnesæmia with clinical symptoms of tetany is now well known, but relatively little is explicitly recorded on hypomagnesæmia of pronounced character without such association. The first workers to draw attention to bovine hypomagnesæmia appear to have been Sjollema and Seekles (1929) and Sjollema, Seekles and van der Kaay (1930).

Although the corresponding clinical condition had long been known on the Continent detailed observations only seem to have been commenced in 1927. The 1929 publication of the Dutch workers, however, provides graphic representation of the relationships between blood magnesium, calcium, and inorganic phosphate, and the association of low serum magnesium with " Kopziekte " or " grass tetany " in distinction to the low calcium of " milk fever " is clearly brought out. An English version of the 1930 paper was also published in the *Veterinary Record* (Sjollema, 1930), and a little later Sjollema (1932) briefly reviewed the Dutch work on occurrence, symptomatology and treatment of " grass tetany," in his article on " Nutritional and Metabolic Disorders of Cattle " in *Nutrition Abstracts and Reviews.*

As the name implies, greatest incidence of " grass tetany " occurred at the time of turning stalled cows out to pasture in spring, and the Dutch workers at first associated the disorder with the flush of early grass, attempting to correlate with high protein content (Sjollema and Seekles, 1933) and high nitrate content (Seekles and Sjollema, 1932, and Sjollema and Seekles, 1933), although without success. The word " voorjaarziekte " used by Dutch farmers obviously implies association of the clinical condition with the early part of the year, and taken in conjunction with Sjollema's data implies a " seasonal hypomagnesæmia."

* Work carried out under the auspices of the " Committee on Nutrition in Relation to Disease," Agricultural Research Council.

The first British work in a parallel direction was undertaken by Dryerre (1932), whose observations established pronounced hypomagnesæmia in cases of so-called " lactation tetany " in England—a term introduced by Lothian (1931) who had reported upon 21 cases from the clinical aspect but without correlation with blood findings. Dryerre's paper also indicates seasonal association with spring pasture and the lower limit for his range of " clinically healthy " animals suggests that there may have been appreciable seasonal variation in serum magnesium irrespective of the appearance of clinical symptoms.

The observations of Blakemore and Stewart (1933) on cows recently turned out to grass showed hypomagnesæmia not only in clinical cases of tetany but also in a proportion of the apparently normal cows of the same herds at the same time. Hopkirk, Marshall and Blake (1933) working on parallel lines in New Zealand also drew attention to the occurrence of hypomagnesæmia in unaffected cows at the time of occurrence of clinical cases of tetany. In the second paper of Blakemore and Stewart (1935) the fall in serum magnesium " shortly after turning out to pasture " in Norfolk, is more specifically evident. The values at the first bleeding on March 28th were normal but at the second bleeding on April 24th were down to two-thirds normal with two values less than half. The duration of lowered magnesium at pasture which, as shown by a third bleeding on May 8th, extended over at least six weeks, coincided with the season during which most cases of " lactation tetany " had been commonly observed in Norfolk. In the earlier Dutch cases (Sjollema, 1930) the association of clinical symptoms with " turning out to pasture " had been even sharper—highest incidence being over the first few days after the change. Blakemore and Stewart (1935) also reported that the feeding of a magnesium-rich mineral mixture prevented the fall in serum magnesium although the pasture already contained ostensible abundance of magnesium, as shown by analysis.

Allcroft and Green (1934) in discussing blood calcium and magnesium of the cow in health and disease also confirmed the association of pronounced hypomagnesæmia with clinically pronounced tetany, but drew attention to its common association with pronounced hypocalcæmia, charting the earlier data of Sjollema in direct comparison with their own to show the frequency with which " milk fever " levels of calcium could be associated with " lactation tetany " levels of magnesium—a point which had not been explicitly stressed by Sjollema although it is very obvious from his data.

In all these observations, however, the general emphasis had been laid either upon the factor of " turning out to pasture " or upon the " flush of early grass " and apart from the occasional appearance of sporadic cases of tetany at other times, even in winter under stalled conditions (Sjollema, 1930), and the hypomagnesæmic tetany of milk-fed calves, apparently first reported by Duncan, Huffman and Robinson (1935), the term " seasonal hypomagnesæmia " usually meant " spring hypomagnesæmia."

The miscellaneous records of this laboratory, however, collected over the years 1933-1937 on the basis of blood samples sent in by veterinary practitioners for differential diagnosis, had revealed numerous instances of hypomagnesæmia associated with autumn rather than spring, and instances in mid-winter amongst animals grazed all the year round, under climatic conditions which precluded " flush of grass " as a factor. When in 1937 an opportunity occurred of studying an unusual outbreak of " mixed hypocalcæmic hypomagnesæmic disorder " on a comparatively large scale in a herd at pasture all winter, occasion was therefore taken to follow blood calcium and magnesium levels throughout a whole year.

The data acquired were unexpected in that they showed a regular seasonal variation in serum magnesium, with a maximum in summer falling to a minimum in winter and rising again through spring to a maximum the following summer, without any clinical symptoms whatever, *i.e.*, a complete dissociation of hypomagnesæmia from spring flush of grass, from " turning out to pasture," from lactation, and from any manifestations of tetany. The observations are being continued on a smaller but more detailed scale over the next two years at least, but the first year's behaviour may be conveniently indicated now. No general discussion, however, need be offered at the present juncture, on the ætiology of clinically manifested hypomagnesæmic tetany ; a highly debatable matter in any case.

Case History.

In March, 1937, a few blood samples were received from F. C. Gillard, M.R.C.V.S., Folkestone, as part of the diagnostic service of the Ministry laboratory. The cases had occurred shortly after parturition in the field amongst a herd of about 200 Hereford cows run entirely out of doors all the year round and had been diagnosed as " atypical milk fever accompanied by convulsions." Altogether 37 cows were affected, all within 21 days of calving and with varying divergence from the conventional picture of milk fever, always in the direction of increased nervous hyperirritability in the early stages. Of these six died with convulsive symptoms before the practitioner reached them, while the remaining 31 recovered after the usual subcutaneous injection of calcium borogluconate. Analyses of the representative blood samples sent in showed the usual low blood calcium of milk fever associated with pronounced hypomagnesæmia, and a request for further samples, both from affected and unaffected cows, was therefore made. By this time, however, the outbreak was over but of six random samples taken about a month after the last clinical manifestation five showed decidedly low levels of serum magnesium with practically normal calcium. No such outbreak had occurred since the owner had taken over the farm about five years before, although occasional sporadic, non-fatal cases of similar " atypical milk fever " had been noted, and the available evidence

therefore suggested a post-parturient hypocalcæmia superimposed upon a seasonal hypomagnesæmia harmless in itself.

A visit was made to the farm at the end of May, 1937, a further selection of blood samples taken, and personal enquiries instituted. The farm itself, " Swanton," lay on the chalk hills about five miles W.N.W. of Dover at an altitude of about 500 feet above sea level, had no farm buildings and had been used entirely for grazing. In all about 200 Hereford cows and 40 cows of assorted breeds were run at pasture the whole year round, suckling their calves for six or seven months until about October, when the latter were weaned and either fattened or kept for replacement of breeding stock. The cows received no supplementary feed except during the worst winter months when hay, grown on the farm, was carried into the fields. This " ranching system " had been successfully carried out since the farm had been acquired in semi-derelict condition about five years earlier, although the owner had finally decided to improve the supplementary feeding, according to climatic conditions and state of grazing, by inclusion of a small ration of concentrates about six weeks before calving.

The owner, Mr. Edward Rice, kindly granted permission to arrange an experiment on 106 of his cows, which were divided into four groups as follows :—

Group A. Fifteen cows from the mixed breed herd to be treated as the whole herd had been previously treated, *i.e.*, confined to grazing and home-grown hay only. This group contained *Br. abortus* reactors and it was agreed that any further reactors would be transferred to it as soon as detected.

Group B. Fifteen Hereford cows to receive the same treatment as Group A but with access to a general mineral mixture (Churn Brand) which the owner had already decided to use with all his cows.

Group C. Thirty-eight Hereford cows to be treated as in Group B until the last six weeks of pregnancy when they might receive supplementary feed according to the owner's wishes. In actual fact, because of the ensuing mild winter, practically no supplement other than hay was necessary, so that Group C did not differ materially from Group B.

Group D. Thirty-eight Hereford cows to be treated as in Group C but in addition to receive an extra source of magnesium in the form of the same mineral mixture fortified with calcined magnesite, giving rather over 50 per cent. magnesium oxide in the whole.

Blood samples were taken every two months commencing June, 1937, and after the sampling in October the animals were divided into their groups although not all physically separated into their respective paddocks until the middle of November when the magnesium supplement was inserted. In the interval two further reactors had been transferred to Group A so that the final numbers in Groups B, C and D became 15, 37 and 37 respectively. Since 15 of the Hereford cows had shown distinctly lower serum magnesium values than the rest at

one or more of the earlier bleedings, these were " seeded " proportionately into Groups B, C and D, actually three in B and six in C and D, in order to make the groups as comparable as possible. The grouping of both " seeded " and " non-seeded " cows was made by allocating individuals in sequence according to horn numbers. Since these serial numbers indicated the time the animals had been on the farm this procedure ensured a fairly even distribution of age classes in an otherwise random selection.

Blood sampling at intervals of two months was continued on the whole 106 cows until December, with the intention of executing more frequent bleedings at the period of most rapid change. The owner, however, then came to the conclusion that the rounding up of the semi-wild beef-breed animals on horse-back, into yards in which they could use their horns, might involve some risk of mechanical abortion as calving date approached, and he therefore limited further bleedings to a selection of 25 cows. This necessitated a reorientation of the experiment in which Group A, the " mixed breed reactors " was abandoned, for reasons which will become evident in discussion, and distribution of the " available 25 " into two groups, " B and C " and D.

By May, 1938, it became desirable to rearrange the experiment for observation over a further two years and with the assistance of special provision recommended by the Agricultural Research Council it became possible to secure continuous control of a group of 24 Hereford cows (with requisite bull) for more detailed work. Of these 24 cows, 19 were available from the 25 used at the February bleeding, the remaining five being selected from other groups. These unavoidable changes during the first year of observation necessitate discussion of the results in sections.

Results.

Serum calcium and magnesium were determined on all blood samples taken, other constituents being occasionally included. Considerations of space preclude tabulation of the complete data but the main essentials in regard to serum magnesium are given in the following tables and charts. Variations in blood calcium need not be included since they were small and erratic and it may merely be stated that no pronounced group changes occurred throughout the year. The average serum values of the 89 Hereford cows in June, August, October and December, 1937, and of the 25 Hereford cows in February, 1938, were 9·9, 9·7, 9·9, 9·4, 9·4 mg. per cent. Ca respectively, but variations in individual behaviour preclude the deduction that group differences such as 9·7 mg. per cent. for August as against 9·4 mg. per cent. for December have any real seasonal significance.

A. *Seasonal Variation in Serum Magnesium.*—The average values in each group and for all 106 cows as a whole, are shown in Table I, expressed as mg. per cent. Mg.

TABLE I.
AVERAGE VALUES FOR SERUM MG IN MG. PER CENT., OF 106 COWS IN FOUR GROUPS.

Date.	*Group A.*	*Group B.*	*Group C.*	*Group D.*	*All Groups.*
June, 1937 ...	—	1·99	2·08	2·02	2·05
August, 1937 ...	2·12	2·22	2·29	2·25	2·24
October, 1937 ...	2·12	1·96	1·96	1·99	1·98
December, 1937	1·88	1·22	1·26	1·60	1·46

No value is assigned to the June sampling for Group A since transfers occurred between June and August, but it may be taken that the average, like Groups B, C and D, was in the region of 2·0 mg. per cent.

At the outset it should be stated that for individual cows arbitrary normal variations from week to week can quite easily cover a range of 0·2 mg. per cent. or 10 per cent., and that it is therefore pointless to express values to the second decimal place when data concern only a few animals. When, however, large numbers, such as the 106 cows in the present experiment, are concerned arbitrary variations tend to cancel out and expression of second decimals becomes justified although conclusions should not be drawn within them. When variations are small the only valid method of assessing their significance is by " frequency distribution," a method adopted in Fig. 2 below.

The data in Table I may be taken as showing a maximum value in August, a slight fall to the June values by October, and thereafter a more rapid fall to December. The December values for Groups B and C are approximately 45 per cent., for Group D 29 per cent., and for " all groups " 35 per cent., below the corresponding August averages. Group A, with a fall of only 11 per cent., shows an unexpected failure to conform with the herd as a whole. These data are graphically represented in Fig. 1.

From Fig. 1 it will be noted that Groups B and C of 15 and 37 Hereford cows respectively, which up to this point had been treated identically, behave in exactly the same way. Group D of 37 similar cows, which had access to the mineral mixture fortified with magnesium oxide for about six weeks before the December sampling, show a distinct stemming of the seasonal fall although the normal summer value is not regained nor the October value even maintained. This point is again referred to below.

The behaviour of Group A, 15 cows of mixed breed including positive reactors to *Br. abortus*, is disconcerting and at the moment inexplicable. They had been intended as a control to possible effects of the commercial mineral mixture supplied to Group B and were the only animals for which permission could be obtained for treatment in the way desired, although it was realised at the outset that they were not perfect controls. Apart from the fact that they were cross-bred instead of pure Hereford and that two or three of them may not have been pregnant, the main observable difference was in the paddock in which they were confined. Although this contained no structural

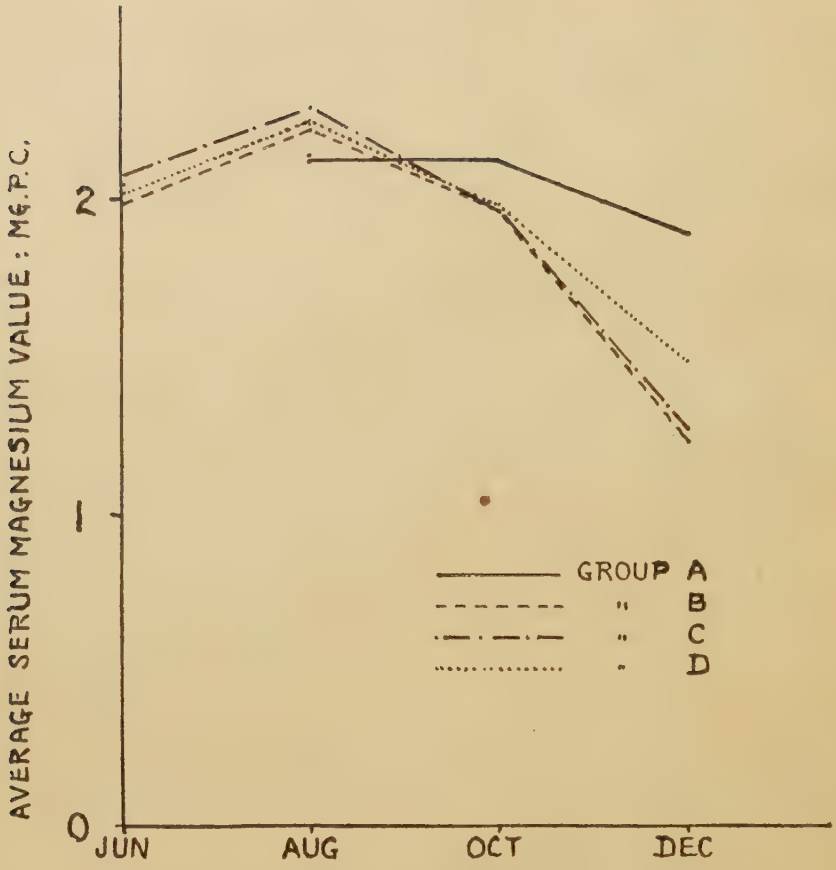

FIG. 1.—Showing seasonal variation in serum magnesium of 106 cows between summer and winter, 1937.

shelter it contained clumps of trees which offered decidedly more natural shelter from adverse weather than did the more exposed paddocks in which Groups B, C and D were confined. Without knowledge of the factors causing the observed seasonal fall in blood magnesium of all the other cows further speculation is unprofitable ; the fact is simply recorded for future guidance and later elucidation.

When considering the behaviour of the 89 cows comprised in Groups B, C, and D, it may be emphasised that the curves of Fig. 1 reflect the individual behaviour of the animals in each group with a high degree of fidelity. At the December bleeding, for instance, only four out of the 89 failed to show values below those of the October sampling ; every one of the remaining 85 showed a definite downward trend.

The seasonal behaviour of the Hereford cows is best shown in the form of histograms displaying the frequency distribution of serum

magnesium values over the four blood samplings. This is done in Fig. 2, in which the 15 cows of Group B are combined with the 37 cows of Group C, since no differential treatment had then been adopted, with the 37 cows of Group D shown separately. Since the total numbers are different, 52 and 37 respectively, comparison is made on a percentage basis.

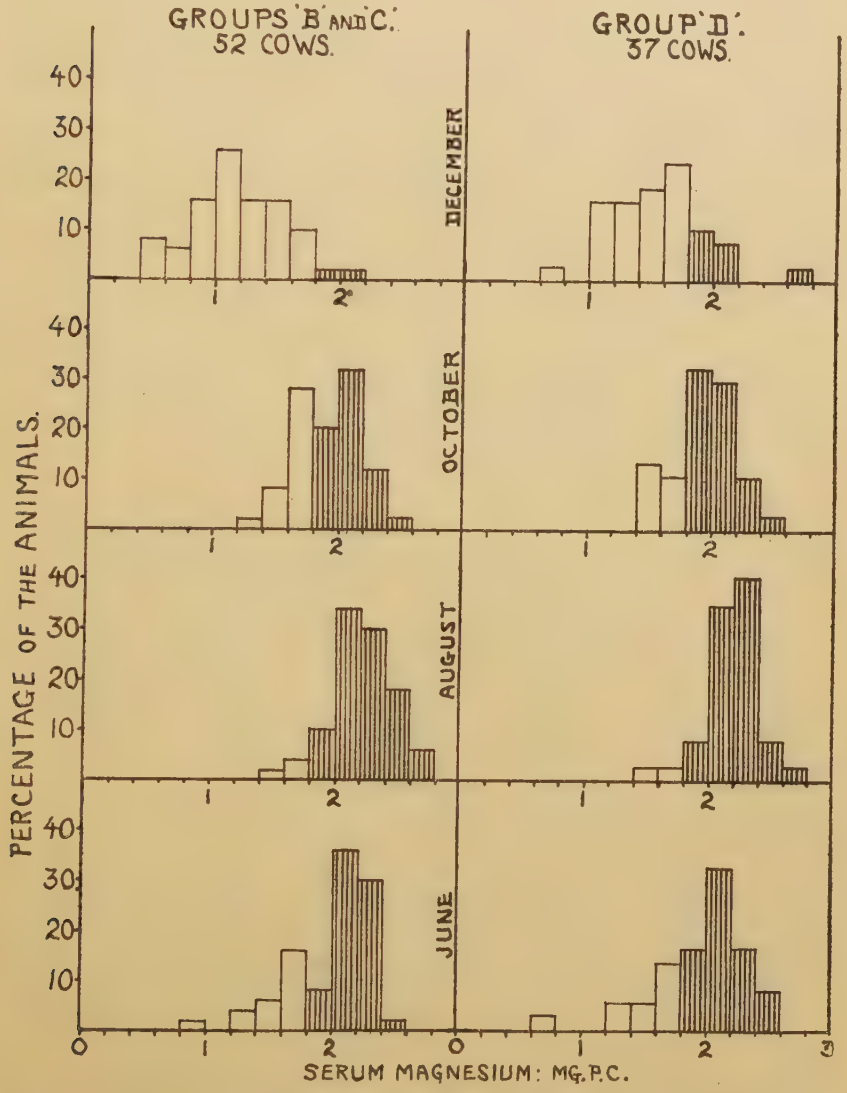

FIG. 2.—Showing frequency distribution of serum magnesium values, at intervals of 0·2 mg. per cent., according to season June, 1937 to December, 1937, in 89 Hereford cows grazing all the year round; Group D receiving magnesia in mineral mixture, Group "B and C" not. All columns representing values over 1·8 mg. per cent., the usually accepted lower limit of the normal physiological range, are shaded.

From Fig. 2 it will be noted that not only is there a decided shift to the right from June to August, decided shift back to the left from August to October, and very pronounced shift from October to December, but also that the columns flatten out between August and December showing an increasing preponderance of low values.

Considering the columns for Group " B and C " it will be noted that in August over 60 per cent. of the cows show values lying between 2·0 and 2·4 mg. per cent. and that some values reach 2·8 mg. per cent. By December very few values even reach the proximity of 2·0 mg. per cent. and no value exceeds 2·2 mg. per cent. Fifty out of the 52 animals have dropped below 1·8 mg. per cent., a figure which is generally regarded as on the borderline of the lower limit of " normal physiological values," while a third of the total have fallen below 1·0 mg. per cent., or *less than half* the commonest frequency values for August, and still lower values, of an order usually associated with " acute lactation tetany " in other parts of the country in spring, are quite common.

Considering the columns for Group D, the general behaviour is much the same although apparently modified by the magnesium supplement to an appreciable extent. Despite the supplement, however, there is the same general shift from values well over 2·0 mg. per cent. in August to values well below in December. The behaviour of Group D is more specifically discussed below.

TABLE II.

SEASONAL VARIATION OF SERUM MAGNESIUM IN 25 HEREFORD COWS OVER JUNE, 1937 TO FEBRUARY, 1938, WITH LARGER GROUP OF 89 HEREFORD COWS UP TO DECEMBER. GROUP " B AND C " HAD ACCESS TO COMMERCIAL MINERAL MIXTURE; GROUP D TO THE SAME MIXTURE ENRICHED WITH MAGNESIA.

Date.	*25 Cows Bled in February,* 1938.	*Cows of Groups B and C in* the 25.	*Cows of Group D in* the 25.	*Average of all the Herefords.*
June, 1937 ...	1·72	1·75	1·71	2·03
August, 1937 ...	2·15	2·12	2·19	2·24
October, 1937 ...	1·83	1·77	1·91	1·98
December, 1937	1·09	0·95	1·30	1·39
February, 1938 ...	1·72	1·69	1·76	—

As already explained it was not possible to continue in 1938 with the full number of cows and that further study was limited to 25 animals by the owner. These were selected from Groups B, C, and D and arranged to include the cows of lowest serum magnesium previously " seeded " into the groups. Their general average should therefore be a little lower than that of the previous larger groups and they might be expected to be more susceptible to the influences controlling seasonal variations, but this was considered to be advantageous from the point of view of future work. The previous records for these 25 cows were then sorted out so as to display their seasonal behaviour as a smaller group. Table II, in which the average of the

previous larger group of 89 Herefords is given in the last column for comparison, shows the behaviour of this group from June, 1937, to February, 1938. The cows of Group D receiving the magnesium-enriched mineral mixture are shown separately but the original intention of differentiating between B and C was abandoned and the animals drawn from these groups are therefore combined as one group.

From Table II it may be noted that the general trend of seasonal change up to December is very similar for the smaller group of 25 as for the larger group of 89. Their general level is lower because of inclusion of a greater proportion of cows of lowest magnesium levels but they are sufficiently similar to be taken as parallel in seasonal behaviour. In the accompanying Fig. 3 they are charted as totals, with the lowest magnesium animals, previously referred to as " seeded," shown separately.

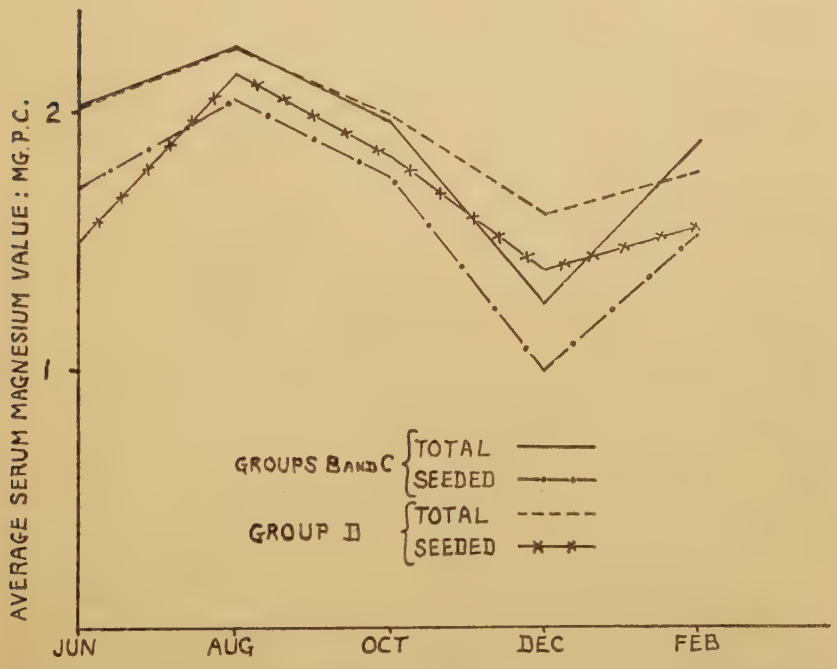

FIG. 3. Showing seasonal variation of serum magnesium in 25 Hereford cows. Group " B and C " had access to commercial mineral mixture ; Group D, access to the same mixture fortified with magnesia.

From Fig. 3 it will be noted that the curves for Group " B and C " and for Group D, " totals," are very similar to those shown in Fig. 1. The curves for the " seeded " members of the groups run roughly parallel at a somewhat lower level. Reduction of the actual numbers to 25 has not affected accurate reflection of seasonal behaviour

and it is therefore legitimate to assume that the subsequent rise in February shown by the smaller group would also have been shown by the larger earlier group to approximately the same extent, *i.e.*, that the smaller group reflects the herd as a whole. This is even more clearly brought out by considering the individual variations of the smaller group amongst themselves, and these may therefore be given in Table III.

TABLE III.

INDIVIDUAL VALUES FOR SERUM MAGNESIUM IN MG. PER CENT. FOR THE "25" COWS.

Number.	*June,* 1937.	*August,* 1937.	*October,* 1937.	*December,* 1937.	*February,* 1938.
7	1·3	1·9	1·7	0·5	1·5
8	1·6	1·9	1·6	1·1	1·1
9	1·8	2·2	1·8	1·2	1·8
10	1·6	2·4	2·1	1·5	2·0
13	2·0	2·4	1·9	1·5	2·0
21	1·7	2·1	1·8	1·0	1·8
22	1·7	2·2	1·9	0·7	1·9
23	1·6	2·0	1·7	1·1	1·7
25	1·6	2·1	1·6	1·4	1·6
26	1·9	2·1	1·7	0·9	1·6
28	1·8	2·3	1·8	1·5	1·9
29	1·8	2·6	1·7	0·9	1·8
31	1·9	2·0	2·0	1·2	1·8
32	2·3	2·3	2·2	1·7	2·2
33	1·0	1·6	1·5	0·8	1·4
40	1·3	1·8	1·3	0·9	1·3
43	1·7	2·1	1·8	1·0	1·4
44	0·8	1·8	1·6	0·8	1·5
106	1·8	2·7	1·8	0·9	1·8
117	1·9	2·4	2·0	1·4	2·0
218	2·2	1·8	1·7	1·2	2·0
231	2·3	2·3	2·2	0·9	1·5
235	1·9	2·3	2·2	1·1	1·9
237	1·9	2·2	1·8	0·5	1·7
249	2·2	2·3	2·3	1·5	1·8
Min.	0·8	1·6	1·3	0·5	1·1
Max.	2·3	2·7	2·3	1·7	2·0
Average	1·72	2·15	1·83	1·09	1·72

Table III shows that of the 25 cows bled in February, 24 showed values higher by anything from 0·2 to 1·2 mg. per cent. than they had shown in December, while the remaining animal showed no change. The behaviour is therefore quite general and since the upward trend is shown by practically every individual it is legitimate to assume that it is representative of all the similar cows on the farm.

Reverting to Fig. 3, and the data of Tables II and III, it is apparent that the seasonal change of serum magnesium consists in a rise through June, 1937, to a maximum in August, a slow fall to October, a sharper fall to a minimum in December, followed by a rise in February, 1938, almost to the preceding June value.

In order to get the further behaviour through the spring months and complete the year 1937-1938, it is necessary to fall back on a

group very similar to, but not quite identical with, the " 25 " of Table II. This is the group rendered available for future work under the special provision recommended by the Agricultural Research Council, already referred to, and consists of 19 cows out of the " 25 " used in February, together with five replacements selected from their previous blood records to match the rest. This group of 24 is therefore, for all practical purposes, also representative of the main Hereford herd on the farm and since individual behaviour is well reflected by group behaviour attention may be confined to the average values charted in Fig. 4.

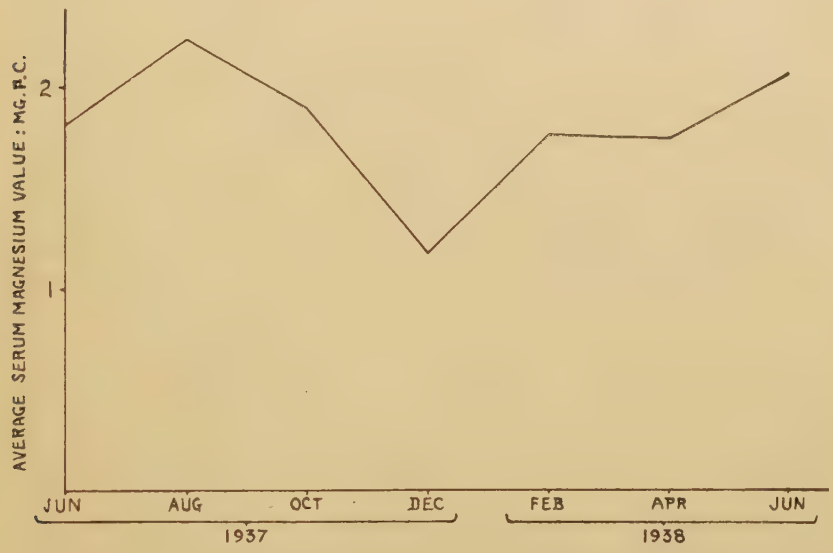

FIG. 4.—Seasonal variations in serum magnesium of 24 Hereford cows over a complete year, June, 1937 to June, 1938.

From Fig. 4, representing a complete year, it appears that the fairly sharp rise from the December minimum through January and February is not followed by any secondary spring fall but by an apparently stationary phase and a gradual further rise to June. In this connection it should perhaps be stated that the incidence of " lactation tetany," and presumably of bovine hypomagnesæmia, throughout England as a whole was very low during this year, the usual " crop of clinical cases " in March, April and May, being almost entirely missing in 1938.

The various data now recorded are far removed from original anticipation. From miscellaneous laboratory records of occurrence of " grass tetany " a moderate autumn fall in serum magnesium of grazing cows was not unexpected, but there was no reason to anticipate a mid-winter minimum. On the contrary the minimum had been expected about March, the time of occurrence of the mishaps which had initiated the investigation in 1937. If such a minimum, instead

of the observed rise to relatively normal values, had been observed in March or April the data would have fallen into line with the Dutch and English observations discussed in the introduction. In those observations, however, the fall coincided not only with the flush of spring grass but also with the change in habits involved in transfer from stall to pasture. In the present case of the Kent farm the cows were out all the year round, minimum blood magnesium values occurred in mid-winter when grass growth was at its lowest ebb, and the magnesium level actually rose over the period when fresh growth of grass was becoming apparent.

In this connection it may be mentioned that in 1934 about 80 animals in the herd of the National Institute for Dairy Research, near Reading, were followed at monthly intervals for a full year, but no significant seasonal variation in blood magnesium was detected. Values in mid-winter in stalls on concentrates and hay were very similar to values for the same cows in mid-summer at pasture. At that institute cows are exercised in the open all the year round and transfer from stall to pasture is gradual, although the date at which the animals are " turned out all night " is of course a demarcation point. In 1938 this change (May 2nd) was followed amongst a smaller group of cows of the institute at short intervals (one to three days) but no pronounced alterations in serum magnesium were detected.

Minimum Individual Magnesium Values.—The other unexpected feature of the data is the occurrence of very low serum magnesium values without any suggestion of clinical symptoms whatever. The records of this laboratory had hitherto dealt with cases diagnosed as " lactation tetany " at the time the blood sample was taken or with samples sent in to confirm suspicion of a hypomagnesæmic state. In such cases magnesium values had generally ranged from 0·3 to 1·0 mg. per cent. with 0·5 mg. per cent. as a common level associated with acute clinical symptoms. Symptoms had occasionally been associated with values as high as 1·4 mg. per cent., presumably from individuals whose normal values were higher than the common average, and Blakemore and Stewart (1935) had even reported levels as high as 1·6 mg. per cent. as associated with lactation tetany.

Comparing the present data the distribution of December values in Fig. 2 indicates an extraordinary preponderance of low values. Of 52 Hereford cows in Groups B and C in the December sampling 12, *i.e.*, 23 per cent., showed values between 0·5 and 0·9 mg. per cent., and no fewer than 29, *i.e.*, 56 per cent., showed values between 1·0 and 1·5 mg. per cent. Despite the abnormally low values prevailing at this time, however, there were no clinical symptoms of tetany, patent or latent, and no evidence of nervous hyperirritability. The procedure of collecting the animals for bleeding provided a severe test involving, as it did, the rounding up on horse-back of animals unaccustomed to handling, driving into yards and then passing one by one through the narrow race in which they were restrained and bled. Each animal finally came under close individual observation

but in no case did driving, yarding and handling precipitate any sign of tetany. The beef-breed cows, run all the year round under semi-range conditions, and never handled except for weaning or bleeding, were never docile or amenable to restraint under the best of conditions but it can be confidently asserted that they were no more difficult to deal with in December than in August when their serum magnesium values were normal.

Effect of Feeding Additional Magnesium.—Comparison of Groups B and C which received an ordinary mineral mixture with Group D which received the same mixture fortified with about 56 per cent. magnesium oxide, shows that until October, before introduction of the magnesia, the group difference is of little significance, but that in December, after six weeks of daily access to the magnesium supplement, the serum magnesium of Group D had not fallen to the same extent as that of Groups B and C. Besides a better average value Group D had also fewer really low numbers. The behaviour is graphically brought out in Fig. 1 and in the histograms of Fig. 2. Although the difference is comparatively small and December values in both cases are much below their normal August values, 1·26 mg. per cent. for the 37 animals in Group C as against 1·60 mg. per cent. for the 37 in Group D, it may perhaps be taken as significant support for the observation of Blakemore and Stewart, cited earlier, that feeding additional magnesium has a preventive effect on the occurrence of seasonal hypomagnesæmia. The mixture in these Kent experiments was offered *ad libitum* and an average quantity of $2\frac{1}{2}$ ozs. per head per day was consumed, equivalent to about 45 grammes MgO, but there is, of course, no guarantee that each animal consumed the same amount, and it is therefore intended to repeat the experiment with more accurately controlled enforced higher magnesium intake. In any case the behaviour is difficult to understand since the pasture grass already contained 0·2 per cent. magnesium expressed on the dry matter, a quantity theoretically abundant if absorbed to the normal extent by the alimentary tract, and further consideration may therefore be postponed until more definite evidence is available. At any rate it should not be inferred without specific proof that magnesium feeding would reduce the incidence of clinical tetany ; offered as a mineral mixture with as high a content as 56 per cent. magnesium oxide it was not even effective in restoring a falling seasonal blood value to normal level.

Of interest in this connection is the fact that although the December minimum was lower for the groups receiving no additional magnesium, the serum magnesium level of the Group " B and C," Fig. 3, caught up with that of Group D between December and February ; whatever factor was operating for the six weeks before the December bleeding apparently vanished during the six weeks thereafter.

Climatic Factors.—As already indicated it is hoped to carry the group of 24 cows, Fig. 4, through at least two more seasons to determine whether the seasonal behaviour of 1937-1938 is characteristic

of the farm, and of individual cows or hereditary strain, or whether the climatic character of the year is involved. From the miscellaneous laboratory records available, and the experience of veterinary investigational officers like F. Blakemore, who has had extensive personal experience of " lactation tetany " in Norfolk, and of practitioners like J. R. Barker, of Hereford, whose practice has kept him in contact with the same farms for many years, the incidence of the hypomagnesæmic clinical condition, associated either with relatively normal calcium or with pronounced hypocalcæmia in the " mixed disorder," varies widely from year to year. Thus February, 1932, was a bad season in Herefordshire leading to the loss of some 500 cows and to the addition of the term " Hereford Disease " to the popular synonyms for the disorder. From that year onwards, until 1936-1937, particularly the period November, 1936 to May, 1937, in Mr. Barker's practice, cases were fewer not only in Hereford but in other counties including Norfolk and Kent. It would appear that some seasons, particularly spring months, are associated with outbreaks of the disease and others, such as the spring of 1938, are singularly free from it. It would appear that climatic factors are involved either in precipitating clinical outbreaks or in defining the conditions which lead to hypomagnesæmia not manifested in clinical form. The history of Swanton farm, relatively free from trouble until the large scale outbreak of 37 cases, or about 19 per cent. of the " susceptible pregnant Hereford cows " on the whole farm, suggests that this particular conflagration was due to a seasonal hypomagnesæmia with minimum serum values just at the time when calving was in full swing. The act of parturition, known to be associated with temporary disturbances in serum levels of calcium and magnesium even in normal cows (Allcroft and Godden, 1934), coming at a period of low serum magnesium level, may possibly have precipitated the outbreak. The calving period in 1938 was about two months later and about five months after the period of minimum serum magnesium level and this may possibly account for the entire absence of clinical cases in the same herd.

There is also evidence, which will be discussed in another connection, that clinical symptoms of tetany are more likely to appear when fall of blood magnesium is *sudden* than when, as in the present case, the change occurs slowly over several months ; that although nervous hyperirritability is related to level of serum magnesium an animal can adjust itself to change if given sufficient time.

Individual Behaviour in Respect of Serum Magnesium.—It is not possible to say with certainty, from the records presented, that any individual cows in the herd were more likely than others to show exceptionally low values of serum magnesium at the period of most pronounced seasonal change. Fig. 3 indicates that the cows " seeded " over the groups on account of low values in earlier tests tended to remain lower throughout the experiment, but it is also true that a number of the other animals showed values just as low at the December blood sampling.

Summary.

(1) Eighty-nine cows of Hereford breed running at pasture all the year round on a Kent farm without shelter and with hay only as supplementary feed in winter, apart from access to a commercial mineral lick, showed a regular seasonal variation in serum magnesium from a maximum in August, 1937, to a minimum in December, with a return in February, 1938, almost to June level of 1937. Winter values were frequently less than half summer values, the extreme overall range being from 2·8 mg. per cent. to 0·5 mg. per cent., but no clinical symptoms of tetany or signs of nervous hyperexcitability were shown.

(2) A smaller group of 15 cows of mixed breed in an adjoining paddock on the same farm, under similar conditions except for greater natural tree shelter, did not show nearly so pronounced a December minimum in serum magnesium level, but no explanation is offered for the discrepancy.

(3) The addition of magnesium oxide to the mineral mixture appeared to alleviate, to a limited extent, the acuteness of the fall in serum magnesium level from October to December, but the difference was abolished by natural return to more normal levels in February. No suspicion of magnesium deficiency is attached to the pasture, the magnesium content of which was 0·2 per cent. expressed on the dry matter of the grass.

(4) The observations are placed on record in relation to the general occurrence of " lactation tetany " or " grass tetany " amongst cows throughout England, mostly during the spring months, but no explanation is offered to account for the seasonal behaviour shown in the data.

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STUDIES IN ANIMAL TRYPANOSOMIASES

Part II.—Disturbances Produced in the Plasma Proteins of *Trypanosoma congolense* and *Trypanosoma brucei*.

By M. H. French, M.A., PH.D.

Tables

(*Continued from p.* 41).

TABLE I.

Plasma protein changes in oxen on a sub-maintenance hay ration during T.congolense infection.

Weeks of Experiment	Ox 2134 (Control)									Ox 3143 (Infected with T.congolense)									
	0	4	8	13	19	27	32	35	44	0	4	8	13	19	20	22	26	32	44
Total N % ...	1.0918	0.9002	0.9929	0.9633	1.0585	1.0575	1.0731	1 0893	1.0626	0.9723	0.9338	0.9596	1.1039	1.1834	1.0756	1.2151	1.1583	1.2080	1.1074
Non-Protein N % ..	0.0343	0.0270	0.0300	0.0344	0 0300	0.0376	0.0339	0.0353	0.0323	0.0338	0.0351	0.0358	0.0388	0.0393	0.0406	0.0362	0.0321	0.0323	0.0354
Total Protein N %	1.0575	0.8732	0.9627	0.9289	1.0285	1.0199	1.0392	1 0540	1.0303	0.9385	0.9037	0.9238	1.0651	1.1440	1.0356	1.1789	1.1262	1.1757	1.0720
Fibrinogen N % ...	0.0582	0.0493	0.0464	0.0466	0.0539	0.0524	0.0568	0.0555	0.0533	0.0517	0.0456	0.0521	0.0631	0.0597	0.0510	0.0650	0.0566	0.0609	0.0556
Euglobulin N % ..	0.0894	0.0932	0.1128	0.0982	0.1220	0.1260	0.1200	0.1228	0.1227	0.0851	0.1463	0.1679	0.1823	0.2336	0.2664	0.1616	0.1430	0.1169	0.1023
Pseudoglobulin N %	0.3954	0.3541	0.3477	0.3465	0.3687	0.3518	0.3615	0.3796	0.3560	0.3738	0.3619	0.3619	0.4020	0.4368	0.4019	0.4429	0.4220	0.4411	0.4099
Total Globulin N %	0.4848	0.4173	0.4605	0.4447	0.4907	0.4778	0.4815	0.5024	0.4787	0.4589	0.5082	0.5298	0.5843	0.6704	0.6683	0.6045	0.5650	0.5580	0.5122
Albumin N % ...	0.5145	0.4066	0.4538	0.4376	0.4839	0.4897	0.5009	0.4961	0.4993	0.4279	0.3499	0.3419	0.4177	0.4139	0.3963	0.5094	0.5046	0.5668	0.5042
Euglobulin as % of Total Protein ...}	8.45	10.67	11.72	10.57	11.36	12.35	11.55	11.65	11.91	9.07	16.19	18.17	17.12	20.42	24.76	13.71	12.70	9.94	9.54
Fibringen as % of Total Protein ...}	5.50	5.65	4.81	5.02	5.24	5.14	5.47	5 27	5.17	5.51	5.05	5.64	5.92	5.22	4.92	5.51	5.03	5.18	5.19
Pseudoglobulin as % of Total Protein}	37.39	37.12	36.12	37.30	35.85	34.49	34.78	36.01	34.55	39.83	40.05	39.18	37.74	38.18	38.81	37.57	37.47	37.52	38.24
Albumin as % of Total Protein}	48.66	46.56	47 55	47.11	47.05	48.02	48.20	47 07	48.37	43.59	38.71	37 01	36.22	36.18	31.51	43.21	44.80	47.36	47.03

Crisis in 20th week.

TABLE II.

Plasma protein changes in oxen on a maintenance hay ration during T.congolense infection.

Weeks of Experiment	Ox 2863 (Control									Ox 2785 (Infected with T.congolense)									
	0	4	8	13	19	25	31	36	44	0	4	8	13	15	18	26	32	36	44
Total N % ...	1.0369	0.9435	1.0009	1.0286	1.1431	1.0780	1.1438	1.1676	1.1818	1.0851	0.8935	0.9793	0.9943	1.0542	1.1015	1.1130	1.0666	1.1021	1.1228
Non-Protein N % ..	0.0286	0.0270	0.0322	0.0333	0.0321	0.0317	0.0323	0.0336	0.0323	0.0522	0.0300	0.0328	0.0318	0.0323	0.0343	0.0323	0.0343	0.0333	0.0342
Total Protein N %	1.0071	0.9215	0.9687	0.9953	1.1110	1.0463	1.1115	1.1340	1.1490	1.0329	0.8635	0.9470	0.9625	1.0219	1.0672	1.0807	1.0323	1.0688	1.0886
Fibringen N % ...	0.0573	0.0565	0.0599	0.0651	0.0658	0.0586	0.0620	0.0585	0.0599	0.0532	0.0489	0.0518	0.0562	0.0555	0.0687	0.0615	0.0579	0.0555	0.0529
Euglobulin N % ...	0.1248	0.1001	0.1115	0.1089	0.1216	0.1170	0.1115	0.1210	0.1238	0.0998	0.1219	0.1358	0.1550	0.1879	0.2027	0.1978	0.1581	0.1472	0.1462
Pseudoglobulin N %	0.3564	0.3331	0.3561	0.3769	0.4067	0.3932	0.4128	0.4152	0.4210	0.3804	0.3285	0.3475	0.3560	0.3569	0.3651	0.3486	0.3364	0.3840	0.3954
Total Globulin N %	0.4812	0.4332	0.4676	0.4858	0.5283	0.5102	0.5243	0.5362	0.5448	0.4802	0.4504	0.4833	0.5110	0.5448	0.5678	0.5464	0.4955	0.5312	0.5416
Albumin N % ...	0.4680	0.4318	0.4452	0 4554	0.5289	0.4775	0.5252	0 5394	0.5443	0.5095	0.3642	0.4119	0.3953	0.4216	0.4407	0.4728	0.4789	0.4821	0.4941
Euglobulin as % of Total Protein ...}	12 39	10.86	11.51	11.04	10.95	11.18	10.03	10.67	10.77	9.48	14.12	14.34	16.10	18.39	18.99	18.30	15.32	13.77	13.43
Fibringen as % of Total Protein}	5.75	6.13	5.77	5.33	4.84	5.60	5.58	5.14	5.22	5.05	5.66	5.47	5.84	5.45	5.50	5.69	5.61	5.19	4.86
Pseudoglobulin as % of Total Protein}	35.39	36.15	36.76	37.87	36.61	37.53	37.14	36 62	36.64	37.08	38.04	36.70	36.99	34 93	34.21	32.26	32.59	35.93	36.32
Albumin as % of Total Protein}	46.47	46.86	45.96	45 76	47.60	45.64	47.25	47.57	47.37	48.39	42.18	43.49	41.07	41.26	41.30	43.75	46.48	45.11	45.39

Crisis in 27th week

TABLE III.

Plasma protein changes in oxen on an ad lib. hay ration during T.congolense infection.

	Ox 2386 (Infected)										Ox 3730 (Infected).			
Weeks of Experiment	0	3	6	9	14	17	20	25	30	39	0	3	6	9
Total N % ...	0.9492	0.9261	0.9847	0.9471	1.0259	1.0821	1.0272	1.0752	1.0864	1.1004	0.9424	0.9083	0.8833	0.9003
Non-Protein N % ...	0.0353	0.0388	0.0365	0.0389	0.0334	0.0323	0.0345	0.0441	0.0324	0.0365	0.0335	0.0335	0.0335	0.0394
Total Protein N %	0.9139	0.8873	0.9482	0.9112	0.9925	1.0498	0.9930	1.0311	1.0540	1.0641	0.9089	0.8743	0.8498	0.8609
Fibrinogen N % ...	0.0426	0.0442	0.0455	0.0451	0.0521	0.0520	0.0506	0.0540	0.0571	0.0605	0.0358	0.0423	0.0421	0.0418
Euglobulin N % ...	0.1029	0.1466	0.1662	0.1545	0.1877	0.2060	0.2180	0.1398	0.1553	0.1232	0.1003	0.1447	0.1785	0.1875
Pseudoglobulin N %	0.3411	0.2878	0.3347	0.3436	0.3911	0.3949	0.3867	0.3504	0.3701	0.3774	0.3327	0.2979	0.3063	0.3165
Total Globulin N %	0.4440	0.4344	0.5009	0.4981	0.5788	0.6009	0.6047	0.4902	0.5054	0.5006	0.4330	0.4426	0.4848	0.5038
Albumin N % ...	0.4275	0.4087	0.4018	0.3680	0.3616	0.3969	0.3377	0.4869	0.4918	0.5030	0.4401	0.3899	0.3229	0.3153
Euglobulin as % of Total Protein	11.26	16.52	17.53	16.96	18.91	19.62	21.95	13.56	12.84	11.58	11.03	16.54	21.00	21.78
Fibrinogen as % of Total Protein	4.66	4.98	4.80	4.95	5.25	4.95	5.10	5.24	5.42	5.68	3.94	4.84	4.95	4.86
Pseudoglobulin as % of Total Protein	37.32	32.44	35.30	37.71	39.40	37.62	38.94	33.98	35.11	35.47	36.61	34.05	36.05	36.74
Albumin as % of Total Protein	46.76	46.06	42.37	40.38	36.44	37.81	34.01	47.22	46.63	47.27	48.42	44.57	38.00	36.62
	Crisis in 20th week and treated with antimosan.										Died in 9th week.			

TABLE IV.

Plasma protein changes in oxen on a good diet of hay and cottonseed during T.congolense infection.

	Ox 3046 (Infected)									Ox 2225 (Infected)								
Weeks of Experiment	0	2	5	10	13	18	22	26	35	0	2	5	10	13	18	22	26	35
Total N % ...	1.0486	1.0096	0.9629	1.1374	1.1672	1.2034	1.2521	1.2369	1.2109	1.0948	1.0788	1.0412	1.1563	1.1369	1.1627	1.2684	1.2626	1.3246
Non-Protein N % ...	0.0343	0.0323	0.0321	0.0353	0.0412	0.0412	0.0323	0.0342	0.0323	0.0343	0.0393	0.0332	0.0363	0.0341	0.0412	0.0335	0.0353	0.0379
Total Protein N %	1.0143	0.9775	0.9308	1.1021	1.1260	1.1622	1.2198	1.2027	1.1786	1.0605	1.0395	1.0080	1.1200	1.1028	1.1215	1.2349	1.2273	1.2867
Fibrinogen N % ...	0.0496	0.0472	0.0489	0.0506	0.0575	0.0516	0.0631	0.0652	0.0625	0.0491	0.0472	0.0495	0.0558	0.0567	0.0562	0.0662	0.0609	0.0630
Euglobulin N % ...	0.1183	0.1249	0.1320	0.1763	0.1929	0.1844	0.1635	0.1519	0.1277	0.1122	0.1295	0.1339	0.1729	0.1892	0.1630	0.1474	0.1316	0.1364
Pseudoglobulin N %	0.4097	0.3830	0.3232	0.4021	0.4035	0.4291	0.4427	0.4407	0.4595	0.4025	0.4103	0.4003	0.4119	0.4021	0.3949	0.4664	0.4726	0.4866
Total Globulin N %	0.5280	0.5079	0.4552	0.5784	0.5964	0.6135	0.6062	0.5926	0.5872	0.5147	0.5403	0.5339	0.5848	0.5913	0.5579	0.6038	0.6042	0.6250
Albumin N % ...	0.4367	0.4224	0.4267	0.4731	0.4721	0.4971	0.5505	0.5449	0.5289	0.4967	0.4520	0.4248	0.4794	0.4548	0.5074	0.5649	0.5622	0.5937
Euglobulin as % of Total Protein	11.66	12.78	14.18	16.00	17.13	15.87	13.41	12.63	10.84	10.58	12.46	13.25	15.44	17.16	14.54	11.94	10.72	10.72
Fibrinogen as % of Total Protein	4.89	4.83	5.26	4.59	5.11	4.44	5.17	5.42	5.30	4.63	4.54	4.92	4.98	5.14	5.01	5.36	4.96	4.90
Pseudoglobulin as % of Total Protein ...	40.39	39.18	34.72	36.48	35.83	36.92	36.29	36.64	33.99	37.95	39.52	39.71	36.78	36.46	35.21	36.96	38.51	37.82
Albumin as % of Total Protein ..	43.06	43.21	45.64	42.93	41.93	42.77	45.15	45.31	44.87	46.84	43.48	42.12	42.80	41.24	45.24	45.74	45.81	46.52
	Crisis in 15th week.									Crisis in 18th week.								

TABLE V.

Plasma protein changes in oxen during T.congolense infections.

	Ox 2855(Infected)			Ox 2864 (Infected)						Ox 3622 (Infected).		
Diet	Maintenance Hay Ration			Good Ration of Hay and Cottonseed						Very good Mixed Ration		
Weeks after Inoculation	0	5	9	0	5	9	10	14	19	0	5	9
Total N %	1.0818	1.0052	1.0612	1.1030	1.0310	1.0104	1.0294	1.0313	1.1956	1.1431	1.0989	1.0444
Non-Protein N %	0.0379	0.0312	0.0343	0.0379	0.0323	0.0433	0.0543	0.0523	0.0823	0.0328	0.0303	0.0402
Total Protein N %	1.0439	0.9740	1.0269	1.0651	0.9977	0.9671	0.9751	0.9990	1.1633	1.1103	0.9939	1.0022
Fibrinogen N %	0.0522	0.0680	0.0644	0.0696	0.0589	0.0546	0.0426	0.0496	0.0518	0.0618	0.0541	0.0344
Euglobulin N %	0.1038	0.1536	0.2036	0.0854	0.1576	0.2028	0.2213	0.1552	0.1451	0.1068	0.2073	0.2570
Pseudoglobulin N %	0.3905	0.3629	0.3952	0.4420	0.3929	0.3479	0.3491	0.3828	0.4499	0.4031	0.3832	0.3779
Total Globulin N %	0.4943	0.5165	0.5988	0.5274	0.5505	0.5507	0.5704	0.5380	0.5950	0.5099	0.5905	0.6349
Albumin N %	0.4984	0.3895	0.3637	0.4681	0.3893	0.3618	0.3621	0.4134	0.5171	0.5397	0.3493	0.3029
Euglobulin as % of Total Protein)	9.93	15.77	19.83	8.02	15.78	20.97	22.70	15.53	12.47	9.61	20.86	25.64
Fibrinogen as % of Total Protein)	5.00	6.98	6.27	6.53	5.90	5.65	4.37	4.97	4.40	5.51	5.44	6.43
Pseudoglobulin as % of Total Protein)	37.37	37.26	34.48	41.50	39.34	35.97	35.80	38.32	38.68	36.29	38.56	37.71
Albumin as % of Total Protein)	47.70	39.99	35.42	43.95	38.98	37.41	37.13	41.38	44.45	48.59	35.14	30.22
	Crisis in 12th week.			Crisis in 9th week and then treated with Antimosan.						Died in 10th week.		

TABLE VI.

Plasma protein changes in sheep during T.congolense infections.

	Sheep W (Infected)						Sheep M (Infected)						
Diet	Poor Hay Ration						Ration of Hay and Cake						
Weeks after Inoculation	0	2	4	6	8	10	0	2	4	6	8	10	17
Total N %	0.9906	0.8706	0.8053	0.8136	0.8044	0.7742	1.0242	0.9453	0.9492	0.9935	0.9538	0.8830	0.8826
Non-Protein N %	0.0273	0.0270	0.0273	0.0316	0.0270	0.0311	0.0314	0.0299	0.0296	0.0296	0.0288	0.0296	0.0301
Total Protein N %	0.9633	0.8436	0.7780	0.7839	0.7774	0.7431	0.9928	0.9154	0.9196	0.9639	0.9250	0.8534	0.8525
Fibrinogen N %	0.0575	0.0488	0.0427	0.0487	0.0449	0.0431	0.0439	0.0479	0.0434	0.0448	0.0511	0.0449	0.0449
Euglobulin N %	0.1079	0.1226	0.1240	0.1410	0.1609	0.1402	0.0840	0.1536	0.1827	0.1688	0.1788	0.1540	0.2035
Pseudoglobulin N %	0.3181	0.3160	0.3086	0.2588	0.2967	0.3241	0.4094	0.3015	0.2881	0.3408	0.3029	0.2959	0.2790
Total Globulin N %	0.4260	0.4386	0.4326	0.3998	0.4576	0.4643	0.4934	0.4601	0.4708	0.5096	0.4817	0.4499	0.4825
Albumin N %	0.4798	0.3662	0.3027	0.3354	0.2749	0.2357	0.4805	0.4074	0.4054	0.4095	0.3922	0.3586	0.3251
Euglobulin as % of Total Protein)	11.20	14.53	15.94	17.99	20.70	18.87	8.47	17.33	19.87	17.51	19.33	18.05	23.87
Fibrinogen as % of Total Protein)	5.97	5.79	5.49	6.21	5.78	5.80	4.93	5.23	4.72	4.65	5.52	5.26	5.26
Pseudoglobulin as % of Total Protein	33.02	37.46	39.67	33.01	38.18	43.61	41.33	35.94	31.33	35.36	32.75	34.67	33.73
Albumin as % of Total Protein)	49.81	42.22	38.90	42.79	35.33	31.72	45.27	44.50	44.08	42.48	42.40	42.02	38.14
	Died in 10th. week.						Died in 18th week.						

TABLE VII.

Plasma protein changes in sheep infected with T.brucei.

	Sub-maintenance Hay Ration.									Productive Ration of Hay and Concentrates.							
	Sheep Umoyo (Infected)			Sheep Dunia (Control)			Sheep Imani (Infected)			Sheep Tumbo (Infected)				Sheep Majani (Control)			
Weeks after Inoculation	0	3	6	0	3	6	0	2	5	0	2	6	10	0	2	6	9
Total N %	1.2006	1.1410	1.0123	1.2386	1.1346	1.1144	1.0206	1.0361	1.0186	1.2474	1.1326	1.0876	1.0208	1.2404	1.1032	1.1144	1.1069
Non-Protein N %	0.0323	0.0323	0.0386	0.0351	0.0326	0.0321	0.0353	0.0351	0.0423	0.0357	0.0323	0.0336	0.0419	0.0349	0.0323	0.0323	0.0333
Total Protein N %	1.1683	1.1087	0.9736	1.2035	1.1020	1.0823	0.9853	1.0010	0.9763	1.2117	1.1003	1.0540	0.9789	1.2055	1.0709	1.0821	1.0736
Fibrinogen N %	0.0562	0.0443	0.0363	0.0581	0.0531	0.0570	0.0520	0.0496	0.0529	0.0649	0.0518	0.0431	0.0551	0.0599	0.0437	0.0505	0.0516
Euglobulin N %	0.1057	0.2957	0.2719	0.1452	0.1288	0.1441	0.0827	0.1389	0.3192	0.1208	0.2257	0.2218	0.3092	0.1432	0.1237	0.1184	0.1501
Pseudoglobulin N %	0.4863	0.4521	0.4039	0.4628	0.4187	0.4081	0.3825	0.3739	0.3889	0.4631	0.4075	0.3680	0.3989	0.4309	0.3831	0.3917	0.4109
Total Globulin N %	0.5920	0.6778	0.6758	0.6080	0.5475	0.5522	0.4652	0.5348	0.7081	0.6839	0.6332	0.5898	0.7081	0.5741	0.5068	0.5101	0.5410
Albumin N %	0.5201	0.3866	0.2615	0.5374	0.5014	0.4731	0.4631	0.4166	0.2155	0.5639	0.4153	0.4211	0.2157	0.5715	0.5154	0.5216	0.4810
Euglobulin as % of Total Protein)	9.05	20.36	27.95	12.06	11.69	13.31	8.39	15.87	32.70	9.97	20.51	21.04	31.59	11.88	11.55	10.94	12.12
Fibrinogen as % of Total Protein)	4.81	3.99	3.73	4.84	4.82	5.27	5.28	4.96	5.42	5.36	4.71	4.09	5.63	4.98	4.55	4.67	4.81
Pseudoglobulin as % of Total Protein)	41.62	40.78	41.48	38.45	37.99	37.71	38.82	37.55	39.83	38.22	37.04	34.92	40.75	35.74	35.77	36.20	38.27
Albumin as % of Total Protein)	44.52	34.87	26.86	44.65	45.50	43.71	47.51	41.62	22.05	46.45	37.74	39.95	22.03	47.40	48.13	48.19	44.80
	Died 1 day later.						Died 1 week later.			Died in 12th week.							

TABLE VIII.

Plasma protein changes in Donkeys infected with Trypanosomes.

	Donkey 17(infected with T.concolense but from 8th week also showing T.brucei						Donkey 22 (Control).				Donkey 3 (Infected with T.brucei.)				
Weeks after Inoculation	0	2	4	6	8	10	0	2	4	6	0	1	3	5	6
Total N %	1.1732	1.0416	1.0976	1.0542	1.0313	1.0121	1.1040	1.1330	1.1214	1.1163	1.0720	1.0416	1.1036	0.9310	0.9604
Non-Protein N %	0.0372	0.0326	0.0411	0.0323	0.0375	0.0306	0.0353	0.0323	0.0343	0.0352	0.0323	0.0341	0.0333	0.0320	0.0435
Total Protein N %	1.1360	1.0090	1.0565	1.0219	0.9938	0.9725	1.0687	1.1009	1.0871	1.0811	1.0397	1.0075	1.0703	0.8990	0.9169
Fibrinogen N %	0.0632	0.0554	0.0561	0.0560	0.0496	0.0415	0.0488	0.0560	0.0531	0.0563	0.0632	0.0593	0.0431	0.0356	0.0362
Euglobulin N %	0.1274	0.2145	0.2424	0.2362	0.2995	0.3311	0.1387	0.1463	0.1311	0.1372	0.1574	0.2453	0.3934	0.3458	0.3311
Pseudoglobulin N %	0.4985	0.4437	0.4621	0.4386	0.3963	0.3379	0.4903	0.4802	0.4768	0.4682	0.4695	0.4783	0.4154	0.3982	0.3961
Total Globulin N %	0.6289	0.6636	0.7045	0.6743	0.6963	0.7190	0.6230	0.6265	0.6079	0.6054	0.6269	0.7241	0.8088	0.7440	0.7272
Albumin N %	0.4459	0.3904	0.2959	0.2911	0.2479	0.2120	0.3974	0.4134	0.4261	0.4194	0.3498	0.2241	0.2184	0.1194	0.1535
Euglobulin as % of Total Protein)	11.21	21.26	22.94	23.11	30.14	34.05	12.41	13.29	12.06	12.69	15.14	24.35	36.76	38.47	36.11
Fibrinogen as % of Total Protein)	5.56	5.49	5.31	5.48	4.99	4.27	4.57	5.09	4.88	5.21	6.08	5.89	4.03	3.95	3.95
Pseudoglobulin as % of Total Protein)	43.88	44.47	43.74	42.92	39.93	39.89	45.85	43.62	43.86	43.31	45.16	47.52	38.81	44.29	43.20
Albumin as % of Total Protein)	39.25	28.78	28.01	28.49	24.94	21.80	37.17	38.00	39.20	38.79	33.62	22.24	20.40	13.28	16.74
	Died on day of last analysis.										Died on day of last analysis.				

TABLE IX.

PLASMA PROTEIN CHANGES IN GUINEA-PIGS INFECTED WITH *T. brucei*.

	2 *Controls.*	*Nos.* 1 *and* 2 (*Infected*).	*Nos.* 3 *and* 4 (*Infected*).
Total N per cent.	0·6904	0·6754	0·6964
Non-Protein N per cent.	0·0147	0·0147	0·0139
Total Protein N per cent.	0·6757	0·6607	0·6825
Fibrinogen N per cent.	0·0366	0·0337	0·0440
Euglobulin N per cent.	0·0566	0·1011	0·1571
Pseudoglobulin N per cent.	0·1831	0·2117	0·2070
Total Globulin N per cent.	0·2397	0·3128	0·3641
Albumin N per cent.	0·3994	0·3142	0·2744
Euglobulin as per cent. of Total Protein	8·38	15·30	23·02
Fibrinogen as per cent. of Total Protein	5·42	5·10	6·45
Pseudoglobulin as per cent. of Total Protein	27·10	32·03	30·33
Albumin as per cent. of Total Protein ...	59·10	47·56	40·20

STUDIES ON FOWL SPIROCHETOSIS.

I.—Immunisation of Chickens with *Spir. gallinarum.*

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Spirochetosis is one of the widespread and economically important diseases of fowls in Palestine. No systematic data are available concerning the morbidity and mortality from this infection. However, the extensive use of arsenicals, both for treatment and prophylaxis, may serve as an index of the loss entailed annually to the chicken breeder.

The immediate practical problem was to ascertain whether it is possible to immunise chickens and protect them against a spirochetal infection. The resemblance of this organism to the relapsing fever spirochete, the immunity of chickens which had recovered from an infection and the successful immunisation experiments reported in the literature indicated that it should be possible to immunise chickens actively. No systematic studies have, however, hitherto been reported on a suitable method of large-scale immunisation and no data are available as to the duration and character of the immunity produced. The object of the studies reported in this and succeeding papers was to obtain an answer to these questions.

Spirochetosis in fowls was first described by Marchoux and Salimbeni (1903) in Brazil and subsequently by Balfour (1908) in the Sudan. The former authors showed that infected blood, heated 5 to 10 minutes at 55° C., or kept 48 hours at room temperature, could be used as an immunising vaccine. Neufeld and Prowazek (1920), immunised chickens with spirochetes dissolved with sodium taurocholate. Similar experiments are reported by Uhlenhuth and Mulzer (1912) with centrifuged spirochetes dissolved in antiformin. Aragao (1911) recommends infected defibrinated blood killed with formaline vapour.

Aside from the difficulty in using blood as a vaccine, there are no data as to the duration or specificity of the immunity. Bonet (1909), Brumpt and Folley (1908), Catanei and Parrot (1926), report that there is cross-immunity between the Brazilian and African strains. Gonder, on the other hand, found that chickens immunised against the Brazilian strain were not immune against the Sudanese and Algerian strains, whereas those immunised against the latter were also immune against the former. Blaizot (1909) claims that young and old chickens react differently to the same strain of spirochete; a spirochete passed through young chickens does not immunise old chickens to infection with a strain passed through old chickens. The strain passed through old chickens does, however, immunise against both. Gonder also observed that the same strain of fowl spirochete passed a long time through rice-fowl no longer gives cross-immunity with the original fowl strain, and *vice-versa.*

It is apparent, therefore, that there are various aspects of the problem still requiring elucidation. In the present paper, we shall present our observations on methods of immunisation, the duration of immunity and the possibility of practical application of the technique developed for large-scale vaccination. In a subsequent paper we shall report experiments bearing on the question of strain and species immunity.

In preliminary experiments, we were able to confirm the results of Aragao with formalised infected blood. The method was, however, obviously inapplicable to large-scale immunisation. Organ suspensions (liver, spleen) also produced immunity but had the same shortcomings as blood. After a series of preliminary experiments, we evolved two methods of immunisation which yielded satisfactory results.

Immunisation with Killed Cultures.

The ideal method appeared to be to apply the procedure followed with bacteria. Attempts were, therefore, made to culture the spirochetes and use killed cultures as vaccine. Two methods of cultivation have been used :—

(1) *Cultivation in Tyrode-serum.*—The simplest method of cultivation, in our experience, is in a medium consisting of 2 parts Tyrode solution and 1 part rabbit serum. The medium is made fresh and distributed into sterile tubes (10 c.c. per tube), closed with sterile rubber stoppers. The cultures are made by adding $\frac{1}{4}$ to $\frac{1}{2}$ c.c. of infected blood during the early stages of the infection. The culture tubes are incubated at 30° C. and, as a rule, maximum growth is attained on the sixth to eighth day.

Only rich cultures (showing 20 to 40 organisms per field on dark-field examination) were used for vaccines. The cultures were collected in large flasks, 0·1 per cent. formalin added, and stored in the ice chest until used. Before use, sterility tests were made in the usual manner.

(2) *Cultivation in Egg Medium.*—Another, more complicated method of cultivation was used ; this method is useful when serial subcultures are desired. The medium was prepared as follows :—

(*a*) Solid base : 1–1·5 c.c. egg albumen added to tubes, slanted and coagulated in an inspissator.

(*b*) A fluid medium was added to cover the entire slant. This medium was prepared as follows :—

100 c.c. Saline-rabbit serum in the proportion of 1 : 5 ;

3 c.c. of a 2 per cent. glycogen in H_2O ;

2 c.c. of 10 per cent. peptone solution in H_2O ;

1 c.c. of a M/15 phosphate buffer solution pH 7·6.

Each ingredient was autoclaved separately and added sterilely to the freshly-prepared diluted serum.

The cultures and vaccine were prepared in the manner described above.

Immunisation Experiments.

The immunisation experiments are summarised in Table I.

Table I.
Immunisation with Killed Culture Vaccine.

Date.	*No. of Exp.*	*Amount Vaccine (c.c.).*	*No. of Chickens.*	*Average Weight (gr.).*	*Interval before Infection.*	*Infective dose of Blood (c.c.) Used.*	*Results of Infection.*
9/7	1	5·0	4	674	2 weeks	0·5	Negative
5/10	2	5·0	6	1,345	2 „	0·25	„
13/12	3	5·0	3	1,563	3½ „	0·5	„
	3a	5·0	3	1,530	3 „	0·5	„
11/3	4	5·0	3	730	4 „	0·25	2 negative 1 infected and died
	4a	2·5	3	608	4 „	0·25	2 negative 1 mild infection, recovered
17/3	5	5·0	2	658	8 „	0·25	1 negative 1 mild infection, recovered
		5·0	1	658	12 „	0·25	Negative
		2·5	1	613	8 „	0·25	„
		2·5	2	613	12 „	0·25	1 negative 1 positive, recovered
16/4	6	5·0	100	3 months old	6 months	0·25	1 tested remained negative
26/5	7	5·0	30	5½ months old	4½ „	0·25	1 tested remained negative
1/6	8	5·0	45	4 months old			

All immunised chickens remained well ; no ill-effects noted.

The vaccine was injected subcutaneously. Since under field conditions, it is extremely difficult to give more than one dose of vaccine, the experimental vaccinations were also confined to one dose.

The vaccine proved entirely innocuous. No reaction of any kind was noted, the birds ate and looked well. The immunity of the treated chickens was tested at various intervals by injecting relatively large doses of infected blood—a severer test than that under natural exposure. In some instances the spirocidal titre of the blood was also examined.

The results showed that the vaccine immunised and that the immunity persisted at least six months. At the same time, it was noted that not all of the birds possessed an equal degree of immunity. Of 28 chickens immunised and tested, three developed mild infections but recovered promptly, while one developed a severe infection and died ; this last was tested only four weeks after the immunisation, thus eliminating time as the responsible factor. Of the 175 birds immunised on farms, none was reported to have contracted an infection and two caught at random and reinfected in the laboratory proved refractory four and a half and six months respectively after the immunisation.

This method may, therefore, be considered practicable and

useful, even though not all of the chickens develop an equally solid immunity.

Immunisation with Immune Serum and Live Spirochetes.

In the course of our experiments with killed vaccine, we noted that the immunity was not always potent. It turned out that vaccines made from later generations of serial subcultures at times failed to produce immunity against the passage strain in fowls. Moreover, even vaccines made from first or second generation cultures did not always induce a solid immunity in all the birds treated. A further consideration was the fact that the cost of the vaccine was higher than desirable.

In view of these considerations we initiated a series of experiments with serum and live spirochetes, or what is generally called the passive-active method of immunisation. This procedure has been used in toxin-antitoxin and virus immunisation, but has not been applied to bacterial or spirochetal infections.

Preliminary studies were required to solve a number of problems. It was necessary to ascertain the minimal amount of immune serum to be injected, the time interval between serum and spirochetes, the danger of infection, the duration and character of the immunity, the effect on the chickens, etc.

A summary of the more significant laboratory experiments is given in Table II, and the data on field immunisation are summarised in Table III.

The procedure finally adopted was as follows: The chickens were injected 0·5 c.c. immune chicken serum and on completion these injections were followed by 0·25 c.c. of fresh, defibrinated, infected blood. In the laboratory experiments, as well as in the early field vaccinations, blood was examined daily for five to seven days to ascertain the presence of infection. Subsequently it was found sufficient to examine only such birds as looked suspicious. At the end of various periods blood was taken from a number of chickens for spirocidal tests and a few were brought to the laboratory and reinfected in order to ascertain their ability to resist an infection.

These results were highly encouraging. Over 50 birds were immunised in the laboratory; of these, two developed an infection during immunisation—both of them in experiments in which the ratio of infected blood to immune serum was one or over. On reinfection at intervals of one to twelve weeks after the immunisation, all chickens proved immune. Injection of immune serum alone no longer protected after an interval of three weeks. In the field, 395 chickens were immunised by this method under normal conditions, and 270 in the midst of an epidemic. In the first group, four chickens showed a transient blood invasion lasting one day, and one succumbed to an infection. In the second group three were found infected, but the infection had probably set in before vaccination.

TABLE II.
IMMUNISATION EXPERIMENTS WITH VARYING DOSES OF IMMUNE SERUM AND LIVE SPIROCHETES.

Date.	*No. of Exp.*	*Amount of Immune Serum (c.c.).*	*Amount of Infected Blood (c.c.).*	*No. of Chickens.*	*Average Weight.*	*Interval before Infection (Weeks).*	*Infection Dose (c.c.).*	*Results.*	*Remarks.*
9/7	1	2·0	0·1	4	762	3	0·25	All negative	The chickens were examined daily for 7 days and no spirochetes found
20/7	2	1·0	0·4	4	740	1	0·25	Negative	Do. do.
27/7	3	1·0	0·5	4	697	2	0·25	,,	1 showed an occasional spirochete one day ; externally well ; weight unaffected
6/8	4	1·0	0·5	2	667	2	0·5	All negative	
		0·5	0·5	2		2	0·5	,,	
		0·25	0·5	2		2	0·5	,,	
15/10	5	0·5	1·0	2	1,230	2	1·0	,,	1 receiving $\frac{1}{4}$ c.c. blood showed spirochetes during immunisation ; recovered.
		0·5	0·5	3		2	1·0	,,	1 receiving $\frac{1}{2}$ c.c. blood showed spirochetes during
		0·5	0·25	3		2	1·0	,,	immunisation and died of infection
18/10	6	0·25	1·0	2	1,350	2	0·25	,,	None infected during immunisation.
		0·25	0·5	2		2	0·25	,,	
		0·25	0·25	2		2	0·25	,,	
10/12	7	0·5	1·0	2	1,300	4	0·25	Negative	None infected during immunisation
		0·5	1·0	2		8	0·25	,,	
		0·5	0·0	2		4	0·25	Positive	Serum alone does not confer immunity after 4 weeks
20 12	8	0·5	2·0	3	1,554	4	0·25	Negative	None infected during immunisation
		0·5	2·0	3		9	0·50	,,	
		0·5	0·0	3		3-4	0·25	Positive	Serum (control) affords no protection
10 3	9	0·30	0·3	8	585	8 (3 ch.)	0·25	Negative	1 died of infection during immunisation ; 2 were lost ; 5 reinfected, 3 after 8 weeks, and 2 after
						12 (2 ch.)	0·25	,,	12 weeks ; all negative
17 3	10	0·3	0·5	8	614	3 months	0·25	All negative	All negative during immunisation. 4 died 1 month after immunisation from cold

Proper controls were used in each experiment in order to test the virulence of the strain and immunity or susceptibility of the chicken used. It will be noted that the infective dose was excessively high, showing that a solid immunity was established.

TABLE III.

MASS IMMUNISATION OF CHICKENS WITH IMMUNE SERUM AND LIVE SPIROCHETES.

Date.	*No. of Exp.*	*Locality.*	*Amount of Immune Serum (c.c.).*	*Amount of Infected Blood (c.c.).*	*No. of Chickens.*	*Age of Birds.*	*Condition of Birds following Immunisation.*	*Results of Immunity Tests.*
16/4	1 1a	K.A. "	0·5 0·5	0·25 0·25	50 100	4 months	Spirochetes found in the blood of 2 chickens on 2nd day and disappeared. Examined daily, 7 days	2 birds were infected 6 months later; 1 was immune, the other had a transient infection lasting 1 day. During 5 months only 16 birds were removed for various reasons. None infected. The laying was normal
26/5	2	M.P.	0·5	0·25	65	4 "	No infection found following the injection. The immunised animals were in a coop infected with ticks. Examined daily 5 days	No spirochetosis developed during 6 months. An epidemic of coryza. Only 11 per cent. of birds removed for various causes—not spirochetosis—as against 33 per cent. in the rest of the flock
1/6	3	P.T.	0·5	0·25	170	5½ "	Only suspicious chickens examined during 5 days; 2 chickens developed mild transient infection; 1 succumbed to severe infection	Place mildly infected with ticks; no spirochetosis during 5 months; condition of birds good; laying normal
26/7	4	N.	0·5	0·25	270	4 "	Vaccination during outbreak of epidemic; 80 birds were weak, 1 of these succumbed to infection. Of 50 chickens examined, 3 of weak ones showed infection; the others were negative. During 2 weeks 12 weak birds were removed though blood was negative	The coop heavily infested with ticks. Ticks collected were infective for chickens in the laboratory. The immunised birds remained free of spirochetosis. The weak birds to the number of 80 were removed from the coop for sale during 3 months following vaccination

This method of vaccination has several advantages. It is simple of execution and not very costly. In infected coops, serum and spirochetes are avilable on the spot. In the face of an epidemic, the passive-action immunisation provides immediate protection to the exposed flock and, at the same time, assures the development of immunity. The prophylactic injection of arsenicals may achieve the former result but not the latter. In view of the insignificant risk and the duration of the immunity (at least six months) this procedure holds out the promise of greatly reducing the losses due to spirochetal infections.

Spirocidal Test as an Indication of Immunity.

It occurred to us that the spirocidal test of the serum might serve as a simple test of immunity. The data given in Tables IV and V indicate that this is true in a general way. It will be noted that spirocidal bodies appeared in the serum of vaccinated chickens whichever method was used. The titre rose gradually and reached a maximum in about three weeks. However, birds tested at longer intervals after vaccination gave variable titres, in some instances as

Table IV.
Spirocidal Tests on Sera from Chickens Immunised in the Laboratory.

No. of Chicken.	*Method of Immunisation.*	*Interval after Immunisation. (Days.)*	*Spirocidal Test : Serum Dilution.* 1 : 100	1 : 500	*Results of Reinfection after (Days.)*	*Spirocidal Test after Reinfection Serum Dilution.* 1 : 500	1 : 1000	1 : 2000	*Interval after Infection. (Days.)*
73	ser. + spir.	42	.	+	i, 88				
74	„ „	42	.	+	i, 88				
98	„ „					—	—	++	11
101	„ „					—	—	+++	11
104	„ „	92	—	+++	i, 95				
105	„ „	92	—	+++	i, 95				
889	serum alone	½ hour	+++						
	ser. + spir.	6 days	+						
		21 „	—	—	i, 56				
893	serum alone	½ hour	+++						
	ser. + spir.	6 days	—	+++	i, 56				
		21 „	—	±					
895	serum	½ hour	+	+++					
	ser. + spir.	6 days	+	+++					
		21 „	—	±					
883	vaccine	6 „	—						
		21 „	—						
885	„	6 „	±						
		21 „	—						
59	„	49 „	.	+	i, 69	—	—	+++	
62	„	49 „	.	++	i, 69	—	—	+++	
64	„	49 „	.	+					

i = immune ; number = after so many days.
. = not tested.
— = complete lysis in 24 hours ; +++ = no lysis.
±, +, ++ = relative number of spirochetes remaining active ; partial lysis.

TABLE V.
SPIROCIDAL TESTS OF SERA FROM CHICKENS IMMUNISED IN FIELD.

No. of Chickens.	*Mode of Immunisation.*	*Interval after Immunisation.*	*Serum Dilution.* 1/10	1/100	
42	ser. + spir.	6	+	+++	Reinfected and found immune
139	,, ,,	6	+	+++	Do.
120	,, ,,	6	—	+	
154	,, ,,	6	++	+++	
171	,, ,,	6	+	++	
Control			+++	+++	Infection positive—died
70	vaccine	6	+	+++	Immune to reinfection
71	,,	6	—	—	
41	,,	6	—	—	
Control			+++	+++	Succumbed to infection
426	ser. + spir.	4½	—	++	Immune to infection
501	vaccine	4½	+	++	Do.
Control			+++	+++	Succumbed to infection
1	ser. + spir.	4½	—	++	Immune to infection
2	,, ,,	4½	—	—	
3	,, ,,	4½	+	+++	
4	,, ,,	4½	++	+++	
Control			+++	+++	Succumbed to infection
301	vaccine	4½	—	—	
327	,,	4½	—	+	
328	,,	4½	—	—	Immune to infection
Control			+++	+++	Succumbed to infection
1a	ser. + spir.	3	—	—	Immune to infection
2a	,, ,,	3	—	—	
3a	,, ,,	3	—	±	

low as 1 : 10 only; all of these birds proved equally immune to infection.

The procedure of the test was quite simple: The serum to be tested was diluted serially with Tyrode solution and 0·4 c.c. added to small tubes. To these tubes were then added one drop of fresh rabbit serum and one or two drops of defibrinated infected blood. The tubes were shaken and placed in the incubator at 30° C. At intervals of ½, 1 and 2 hours, drops were withdrawn and examined under the dark field. The tubes were then left at room temperature and again examined at the end of 24 hours. Controls were set up with normal serum. The degree of agglutination and dissolution of the organisms served as a measure of the activity of the serum.

SUMMARY.

Experiments are presented showing that it is possible to immunise chickens against spirochetal infections by two methods :—

(1) One procedure consists in the use of formalised cultures; 1 dose of 5 c.c. is sufficient to produce immunity.

(2) The second method utilises immune serum plus live spirochetes. The serum is given first (0·5 c.c.) followed shortly by an injection of 0·25 c.c. of defibrinated infected blood.

Both methods have been employed in the field with promising results. The second procedure is particularly applicable during epidemic outbreaks since it affords both passive and active immunity.

The vaccines induce the production of spirocidal substances. These disappear progressively from the circulation but their disappearance does not indicate loss of immunity. Thus far we have found that the immunity lasts at least six months.

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STUDIES ON FOWL SPIROCHETOSIS

II.—Presence of Serologically Differentiated Types of Spirochetes.

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In the course of our studies on the immunisation of chickens with spirochetes, we noted that strains derived from different sources were serologically distinct. The literature contains very few references bearing on this aspect of the problem and the reported results appear to be contradictory. Bonet (1909), Brumpt and Foley (1908), and Catanei and Parrot (1926), report cross-immunity between the Brazilian and African strains. Gonder, on the other hand, found that the Brazilian strain immunised only against itself and not against the Sudanese strain, although the latter immunised against both. Blaizot (1909) reports that young and old chicken behave differently with respect to the same original strain of spirochete : the passage strain in young chicks cannot immunise old chickens against an infection with the same strain passaged through old chickens ; the latter, however, immunise against both strains. Gonder also found that the same strain passed respectively through chickens and rice-fowl became immunologically distinct. So long as the question was purely theoretical these apparently contradictory findings have received little attention. In connection with our studies on the practicability of mass immunity, this aspect of the problem also assumed practical significance. Consequently we set out to study the serological relationship of spirochetes derived from various parts of the country.

Strains of spirochetes were obtained from Kfar Gileadi in the North, from Afuleh (in the Valley of Jezreel), from Jerusalem, and from Gedera and Naaneh (in Judea). The Jerusalem strain was obtained from an infected chicken, the others were isolated from ticks collected at the places mentioned. In Afuleh, collections of ticks were obtained from three different farms.

The relationship of the different strains was determined by the agglutinating and spirocidal action of strain-specific immune sera, and by cross-infection of immunised chickens as well as chickens which had recovered from an infection. The two sets of tests gave comparable results.

It is of interest to note that the five places yielded four distinct strains. The strains from Kfar Gileadi, Jerusalem and Naaneh were serologically distinct, and of the three strains obtained from Afuleh, one was identical with that from Naaneh, one with that from Kfar Gileadi, while the third differed from all the other strains. The Gedera strain was identical with that from Naaneh.

The results of the various tests and infection experiments are summarised on next page.

Table I.
Cross-Spirocidal Tests with Strain Specific Immune Sera.

Strain.	*Serum (Dilutions).*													
	(*a*) *Jerusalem.*					(*b*) *Kfar Gileadi.*					(*c*) *Naaneh.*			
	100	500	1,000	2,000	4,000	100	500	1,000	2,000	4,000	100	500	1,000	2,000
Jerusalem.														
Examined after :										*				
2 hours	—	—	±	+++		+++	+++	+++	+++		++	+++	+++	
24 „	—	—	—	—		+++	+++	+++	+++		++	+++	+++	
Gileadi.														
Examined after :														
2 hours	+++	+++	+++			—	++	+++	+++		++	+++	+++	
24 „	+++	+++	+++			—	±	+++	+++		++	+++	+++	
Jerusalem.														
Examined after :														
2 hours	—	—	±	±										
24 „	—	—	—	—	±									
Gileadi.														
Examined after :										*				
2 hours						—	—	+	++					
24 „						—	—	—	—	+				
Naaneh.														
Examined after :														
2 hours	+++	+++	+++			+++	+++	+++			—	—	+	+++
24 „	++	++	+++	+++		++	+++	+++			—	—	±	++

\+ ++ +++ = No. of spirochetes per field = partial or no lysis.
— = Absence of spirochetes = complete lysis.
* Two different antisera were used, one more potent than the other.

A.—Cross-Spirocidal Test.

The spirocidal test was made in the following manner. Specific immune serum was obtained from chickens which had recovered from an infection. As a rule the spirocidal titre of such a serum against the homologous strain was 1 : 2,000 to 1 : 4,000. Serial dilutions of the immune serum were made in tyrode solution containing 10 per cent. normal rabbit serum. Each tube contained 0·5 c.c. of the diluted immune serum, to which were added one or two drops of defibrinated infected blood. The tubes were incubated for two hours at 30° C., and then for 24 hours at room temperature.

Specific anti-sera for the Jerusalem, Kfar Gileadi and Naaneh strains were tested against the three strains. The results are summarised in Table I. The distinctive characters of these strains is apparent.

The strains obtained from Afuleh and from Gederah were tested with the specific sera obtained with the three identified strains. The results are summarised in Table II. It will be noted that the Gederah and one of the Afuleh strains were identical with the Naaneh strain, that another of the Afuleh strains was completely lysed by the Gileadi serum, and that the third Afuleh strain was not affected by any of the sera used.

Table II.

Spirocidal Action of Specific Known Sera against Different Unknown Strains.

Strains.	*Sera (Dilutions).*								
	Jerusalem.			Kfar Gileadi.			Naaneh.		
	100	500	1,000	100	500	1,000	100	500	1,000
Af.M.	+++	+++	+++	—	—	—	+++	+++	+++
Af.H.	+++	+++	+++	+++	+++	+++	+++	+++	+++
Af.W.	+++	+++	+++	+++	+++	+++	—	—	—
Ged.	+++	+++	+++	+++	+++	+++	—	—	—

Note.—24 hour readings are given.

B.—Cross Infection Experiments.

These serological tests were repeated several times with the same results. There can, therefore, be little doubt as to the serological specificity of the strains. It remained to be determined whether antigenic differences would also manifest themselves in pathogenic specificity in cross infection experiments.

Two types of experiments were carried out. In one series a number of chickens were immunised with a given strain ; these were then subdivided into groups each of which was infected with another strain. In another series chickens which had recovered from an infection with one strain were infected with another strain to ascertain whether an infection with one strain produces immunity against a heterologous strain. In all about 100 chickens were submitted to a single or double cross infection. The results are summarised in Tables III, IV and V.

TABLE III.

CROSS INFECTION TESTS AFTER IMMUNISATION AGAINST THE JERUSALEM STRAIN.

		Immunisation with		*Homologous Infection.*			*First Cross Infection.*				*Second Cross Infection.*			
Exp. No.	*No. of Chickens.*	*Serum + Infected Blood*		*Time Interval.*	*No. of Chickens.*	*Results of Infection.*	*Time after Specific Infection.*	*No. of Chickens.*	*Infecting Strain.*	*Results.*	*Time Interval.*	*No. of Chickens.*	*Infecting Strains.*	*Results.*
		c.c.	*c.c.*											
1	4	0·3	0·3	3 months	4	Neg.	10 days	4	Gileadi	1 neg. 3 pos. (1 died)	3 weeks	2	Naaneh	Both pos. (1 mild ; 1 moderate)
2	3	0·3	0·5	3 „	3	„	10 „	3	Gileadi	1 neg. 2 pos. (1 died)	3 „	2	Naaneh	Both pos. (1 died)
3	2	0·5	0·25				1 month	2	Naaneh	Pos.	7 „	2	Gileadi	1 neg. 1 pos.
4	2	0·25	0·25	5 weeks	2	„								
4a	2	0·25	0·25				5 weeks	2	Gileadi	pos. (mild inf.)				
5	3	0·5	0·25	3 „	3	„								
	2	0·5	0·25				3 „	2	Gileadi	pos.				
	2	0·5	0·25								3 „	2	Naaneh	1 neg. 1 pos.(died)
6	9	0·5	0·25	3 „	3	„								
							3 „	3	Gileadi	1 neg. 2 pos.				
											3 „	3	Naaneh	pos. (heavy; 1 died)
Total				Jerusalem	15	All neg.	Gileadi	16		4 neg. 12 pos. (2 died)	Naaneh	11		1 neg. 10 pos. (3 died)

TABLE IV.
CROSS IMMUNITY AFTER IMMUNISATION AGAINST KFAR GILEADI STRAIN.

		Immunisation with		*Homologous Infection.*			*First Cross Infection.*				*Second Cross Infection.*			
Exp. No.	*No. of Chickens.*	*Serum + Infected Blood* c.c.	c.c.	*Time after Immun.*	*No. of Chickens.*	*Results. of Infection.*	*Time Interval after Immun.*	*No. of Chickens.*	*Infecting Strain.*	*Results.*	*Time Interval after Infection.*	*No. of Chickens.*	*Infecting Strain.*	*Results.*
1	2	0·5	0·25				3½ weeks	2	Naaneh	Pos.	2 weeks	2	Jerusalem	Pos. (1 died)
2	3	0·5	0·25				3½ „	3	Jerusalem	Pos.	2 „	3	Naaneh	Pos. (1 died)
3	5	0·25	0·25	5 weeks	3	Neg.	5 „	2	Jerusalem	Pos.				
4	6	0·5	0·25	3 „	2	„								
							3 „	2	Jerusalem	Neg.				
											3 „	3	Naaneh	Pos. (both died)
5	3	0·5	0·25	3 „	3	Neg.								
							3 „	3	Jerusalem	Pos. (1 mild)				
											3 „	3	Naaneh	Pos. (1 died)
Total				Gileadi	8	All neg.	Jerusalem	12		2 neg. 10 pos. (1 died)	Naaneh	10		All pos. (4 died)

TABLE V.
CROSS IMMUNITY TESTS AFTER IMMUNISATION AGAINST NAANEH STRAIN.

		Immunisation with		*Specific Infection.*			*First Cross Infection.*				*Second Cross Infection.*			
Exp. No.	*No. of Chickens.*	*Serum + Infected Blood* c.c.	c.c.	*Time after Immun.*	*No. of Chickens.*	*Results.*	*Time after Infection.*	*No. of Chickens.*	*Infecting Strain.*	*Results.*	*Time Interval.*	*No. of Chickens.*	*Infecting Strain.*	*Results.*
1	8	0·5	0·25	5 weeks	2	Neg.	5 weeks	3	Jerusalem	1 neg. 2 pos. (1 died)	5 weeks	3	Gileadi	2 neg. 1 pos.
2	21	0·5	0·25	2 „	6	„	2 „	7	Jerusalem	2 light 3 heavy (2 died) 2 neg.	2 „	7	Gileadi	2 pos. (heavy, died) 5 neg.
Total				Naaneh	8	All neg.	Jerusalem	10		3 neg. 7 pos. (3 died)	Gileadi	10		7 neg. 3 pos. (2 died)

An examination of the tables shows that in general the strains are specific in their behaviour; each strain produces a solid immunity against an infection with the homologous strain. At the same time, there is also a varying degree of cross protection. The Jerusalem and Gileadi strains afford mutual cross protection to about 20-25 per cent. of the chickens infected with these strains. Neither of them affords, however, any appreciable protection against the Naaneh strain. This latter strain is the most virulent of those studied, and it is interesting to note that, although it produces a considerable cross protection against the other two strains, they in turn do not protect against it. Of ten chickens immunised with the Naaneh strain and subsequently infected with the Jerusalem strain three were negative and seven developed an infection (three of them fatal). Of the similar lot of ten chickens infected with the Gileadi strain seven were negative and only three developed an infection (two of them fatal).

Similar results were obtained with chickens that had recovered from an infection with one strain and were subsequently re-infected with one of the other strains. It is apparent, therefore, that both cross infection and serological tests demonstrate the existence of specific antigenic types of spirochetes. It is not unlikely that in nature the degree of cross immunity following an infection is greater than that obtained in the laboratory experiments for the obvious reason that we have employed rather heavy infective doses. It is important, however, to know that strain specific types occur, in order to account for possible ineffectiveness of immunisation which may be due to a heterologous infection. In all of the experiments presented in this paper the immunity against homologous strains has proved to be solid even when heavy infective doses were used.

Summary.—Experiments are reported demonstrating that there exist in Palestine a number of strains of fowl spirochetes which are antigenically distinct. Chickens immunised with a given strain are definitely immune to an infection with the homologous strain, but remain susceptible to an infection with a heterologous strain.

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THE RESISTANCE OF THE SWINE FEVER VIRUS TO PHYSICAL AGENCIES AND CHEMICAL DISINFECTANTS*

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The consideration of the problems involved and the description of the work done may conveniently be divided into three parts. These three parts are:—

I. Viability of the swine-fever virus in acid and alkaline media.
II. The survival of the virus when exposed to atmospheric influences.
III. The resistance of the virus to chemical disinfection.

I.

The viability of a number of viruses in acid and alkaline media has received considerable attention but, so far as can be ascertained the behaviour of the swine-fever virus in this respect has never been investigated. Accordingly, experiments were devised to test the resistance of this virus to changes in the hydrogen-ion concentration on both sides of neutrality, in the hope of discovering something which might be helpful in choosing a disinfectant.

At first, a defibrinated blood, haemolysed by diluting 1-10 with distilled water, was used, but this was discarded later in favour of serum diluted 1-10 with distilled water. After setting the pH to the required point, and waiting for a determined interval the blood or serum was inoculated in amounts of 1 c.c. into susceptible pigs. It was known by actual test that this was many times the minimal infective dose. For storage purposes—to allow of use for later inoculations—treated serum was filtered through Seitz filters and kept in the cold store.

For setting the pH, a quantity of 50 c.c. of blood or serum was placed in a small beaker and the electrodes of a Cambridge pH-meter were immersed in it. The dials of the instrument were turned to the pH required and, with constant stirring, sufficient hydrochloric acid or caustic soda was added to bring the indicating galvanometer to the zero point. Both reagents were chemically pure. The strength of the reagents varied according to the pH required so as to cause as little increase as possible in the volume of the blood or serum. The Cambridge instrument allows rapid and accurate setting of the pH and has the additional advantage that dyes or other substances which might injure the virus are not required. The only possible chemical contaminant is potassium chloride from the calomel electrode and there is nothing to indicate that it is other than inert towards the virus.

* This investigation was carried out with the aid of a grant made to the Agricultural Research Council by the Imperial Chemical Industries, Ltd.

It was noted that in some instances there was considerable precipitation of the proteins of the serum, but the virus was shown still to be present in the liquid after the removal of this precipitate by filtration.

It was originally intended to test the range of pH 3·0 to pH 13·0, but in two cases excess of reagent was added, bringing the two samples concerned to pH 1·4 and pH 10·8. These two samples of serum were retained and inoculated as a matter of interest, and it was fortunate in the case of pH 1·4 sample because that was the only concentration on the acid side at which the virus was affected.

The results are shown in Table I. It will be seen that the virus is very resistant to changes in hydrogen-ion concentration. In this property is is similar to the majority of other viruses. Notable exceptions are the viruses of foot-and-mouth disease and of vesicular stomatitis.

II.

It must often happen that virus-containing excretions or secretions become absorbed into materials such as bricks, wood, etc., in contact with infected animals, and in the literature there is considerable speculation on the subject but little actual work has been done. Geiger (1931), recognising the danger of transferring swine-fever infection through the custom of delivering food in sacks which are later returned for refilling, found that dried blood remained virulent for 20 days, and faeces and urine for seven days, on pieces of sacking. In spite of these results he concludes that infection is unlikely because of the great dilution of virus in the food which has come into contact with contaminated sacks and because of the known difficulty of infecting pigs by ingestion. In the present investigation a few experiments were carried out to test the susceptibility of pigs to infection by routes other than the subcutaneous and intravenous. One pig was allowed to ingest 10 c.c. of a 5 per cent. spleen suspension (see Part III) in a small quantity of food and did not become infected. Two other pigs were tested, one by injecting a few cubic centimetres of spleen-faeces mixture into the nasal cavities and the other by dropping a little of the same material into the eye. In both cases the pigs remained healthy, although a minute fraction of a cubic centimetre would have certainly infected by the subcutaneous route. All of the pigs were later proved by subcutaneous inoculation to be fully susceptible.

The materials selected for test were soft red brick and chopped hay. Brick slabs two inches square and ¼ inch thick were autoclaved, dried over calcium chloride *in vacuo* and, after soaking in 5 per cent. spleen suspension, again dried. In the case of the hay, portions of clean hay each weighing two grammes were chopped with scissors and placed in small jars and 2 c.c. of 5 per cent. spleen suspension poured into each jar. After thorough soaking the hay was dried in the same way as the pieces of brick.

At first it was decided to determine how long the virus would

survive on brick and hay when exposed to the air. For this purpose a louvred box was made, so that while exposed to the air the hay and brick were protected against light and rain. Every seven days from the time of first exposure, one piece of brick and one sample of hay were removed and ground in a mortar. The brick was easily broken down to a fine sandy powder, and after the addition of about 20 c.c. of saline, the whole of the suspension was filtered through coarse paper in a Buechner funnel, using a vacuum pump to assist filtration. The whole of the filtrate was injected into a pig. The hay was treated similarly but sand was used to help the grinding and extraction. The results are given in Table II.

Disinfection of Brick.

Square slabs of dry infected brick, prepared as described above, were immersed in 200 c.c. of hypochlorite disinfectant for 15 minutes, well washed in running water for one hour, ground to a powder in about 20 c.c. of saline and filtered through paper in a Buechner funnel. The whole of the filtrate was injected. It was noticed that the treated brick still smelled strongly of chlorine after washing. The results are given in Table III. It will be noted that in spite of the large excess of disinfectant present and the fact that the infected spleen was attached to inert inorganic material, the strength of hypochlorite required to destroy the virus was exactly the same as that for spleen suspension alone.

III.

There is little accurate information available about the destruction of the swine fever virus with disinfectants. The only experimental animal available is the pig and the most elaborate precautions must be taken to exclude accidental infection. A difficulty is the selection of a suitable technique. Investigators have attacked the problem in different ways. The earliest efforts were simply to mix infective blood or secretion with an appropriate amount of disinfectant and to inject the mixture into a susceptible pig after the lapse of the required time. This method is open to the serious objection that the action of the disinfectant continues for an unknown time at the site of injection. In large scale experiments Miessner and Geiger (1930), and later Uhlenhuth, Miessner and Geiger (1933), devised a new method. They prepared paper-thin slices of kidneys showing petechiae from pigs infected with swine-fever. These slices were immersed in the disinfectant for the required time, well washed in water, ground in a mortar and injected into pigs. Formalin 2·5 per cent., lysol 6 per cent., Caporit 2·5 per cent., and a number of proprietary disinfectants failed to destroy the virus in one hour. The most effective agent was caustic soda, either alone or in combination with milk of lime. Neither 2 per cent. nor 3 per cent. caustic soda killed the virus completely, but weakened it considerably. Later, these same workers tested a number of newer caustic soda preparations such as Duramin and Trosilin. These are proprietary preparations which form caustic soda when dissolved in water: Trosilin also

contains chlorine. Both of these substances were more effective than pure caustic soda. Caustic soda in any form has the great advantage that it will penetrate and dissolve dry organic material, but the considerable disadvantages of being dangerous to handle, and of having a corrosive action on the skin of animals placed in recently disinfected premises. Uhlenhuth and his co-workers came to the conclusion that 2 per cent. caustic soda in combination with 5 per cent. milk of lime—the milk of lime is added to show the distribution of the disinfectant—was the best available disinfectant for use in swine fever. Helm and Wedemann (1932) carried out field experiments using naturally infected railway wagons. Pigs were infected and placed in isolated railway wagons. When the pigs died or were slaughtered one wagon was thoroughly disinfected and the other left untouched. Susceptible pigs were immediately placed in the disinfected and non-disinfected wagons. Great difficulty was experienced because pigs placed in infected wagons often failed to contract swine fever. They found that, at summer temperatures, 2 per cent. caustic soda plus 5 per cent. milk of lime killed the virus in one hour. At moderate temperatures 24 hours was necessary and at freezing temperatures 24 hours was insufficient. The authors rightly point out the great importance of a satisfactory disinfectant for railway wagons which have to be used continuously for animal transport. David (1931) carried out similar experiments using infected food troughs. The results were irregular; pigs feeding from grossly infected troughs (smeared with virulent blood) often did not become infected, whereas other pigs sometimes became infected from troughs apparently disinfected.

It will be obvious from the foregoing that a satisfactory test for disinfectants for use against the swine-fever virus has not yet been found. The kidney slice method is expensive and, because of variation in the texture of the kidney slice, is bound to give variable results. The contact methods are unsatisfactory because of the irregular way in which infection is picked up. Bearing in mind the drawbacks of the methods already used, an attempt was made to evolve a technique which would be easy to carry out and at the same time be easy to repeat. As a source of virus dried spleen was selected. A number of spleens were removed aseptically from pigs infected artificially six days previously with a potent virus, minced and quickly dried over calcium chloride *in vacuo*. As it is known that dried virus retains its virulence unaltered for long periods, a sufficient quantity of spleen was dried to cover the whole range of experiments. The whole procedure of removal of spleens, drying, etc., was checked at every stage to exclude bacterial contamination. The strain of virus used was very virulent, and often killed pigs in six days and, with very few exceptions, pigs ceased to feed on the third day after inoculation.

At first a 5 per cent. suspension of dried spleen was used to test the disinfectant. A weighed quantity of dried spleen was carefully

ground to a fine powder in the dry state and suspended in distilled water. This suspension was then filtered through glass wool to remove gross particles. Later, it was decided to use something approximating more to natural condition. A quantity of pig's faeces was autoclaved, dried and ground to a coarse powder in a mortar. For the actual tests a 5 per cent. spleen suspension was added to a weighed quantity of dry faeces in the proportion required to restore the original moisture content of the faeces. It was found that normal pigs' faeces at this laboratory contained approximately 79 per cent. of water. After mixing the spleen suspension and faeces the mixture was allowed to stand for one hour to ensure thorough soaking. The result was a bacteriologically sterile virus-containing faeces which could be inoculated without fear of undesirable consequences. The whole of the infected faeces was placed in a small sterile beaker provided with a cover, and an amount of disinfectant, equal in volume to that of the spleen suspension used, was added and the whole thoroughly mixed. The mixture was then carefully transferred to a fresh beaker and left to stand for the required time. This transference after the addition of the disinfectant is essential, because, in mixing, some virus may adhere to the upper part of the sides of the beaker and be removed from the action of the disinfectant. After the lapse of the required time the mixture was filtered rapidly through a layer of glass wool in a Buechner funnel, using a vacuum pump to hasten the filtration. The filtrate was injected as quickly as possible, at the most 2-3 minutes, after filtration was completed. A considerable proportion of this work was concerned with hypochlorite solution and there was no disadvantage in injecting the filtrate subcutaneously as hypochlorites are rapidly destroyed in the presence of organic matter. In all other cases the filtrate was injected intravenously. In some instances symptoms of poisoning developed, but, with one exception, they were of no importance. In this one case 10 c.c. of spleen-faeces mixture containing phenol caused the death of the pig in a few minutes.

The results are given in Table 3. It is necessary to add that, in those experiments in which a lethal concentration was found, only that concentration and the lower one, which failed to kill, are given.

It will be seen that the time of exposure—15 minutes—is rather short, but for use in connection with swine fever a disinfectant must act rapidly, because it is only in those instances in which rapid destruction of the virus is required that disinfection is practised. There was no advantage in prolonging the time beyond 15 minutes when testing the hypochlorite solution, because the activity of the solution is probably lost long before the lapse of this time (see footnote.) To obtain comparable results the other disinfectants had to be allowed to act for the same length of time. A too short exposure to the disinfectant is undesirable because the irreducible time required for filtration and injection then forms a large part of the total exposure-time.

The following information was supplied by the Technical Service Department of Imperial Chemical Industries Ltd. :—

" Twenty-one grammes of standard dried swine faeces were mixed with 79 grammes of water and allowed to soak for one hour. Meanwhile a 12·5 per cent. solution of Chloros—that is, one volume of the latter diluted to eight volumes with water—was prepared and was found to contain 1·65 per cent. available chlorine (weight in volume). Seventy-nine grammes of this solution were added to the suspension of swine faeces and the mixture was gently shaken. Portions were removed, weighed, and tested for available chlorine content at known intervals of time, and the results were calculated to the equivalent weight present in the whole original amount of the mixture (179 grammes). The following results were obtained :—

Time (min.)	*Grammes available chlorine*	*Time (min.)*	*Grammes available chlorine*
0	1·27 (calculated)	25	0·18
5	0·24	30	0·16
10	0·21	90	0·10
15	0·20	18 hours	0·02
20	0·19		

All of the experimental pigs came from disease-free stock and were kept in isolation at the laboratory for some time before inoculation. They were all of approximately the same weight and size (60-80 lbs.). After inoculation they were kept in separate steel sties and were never handled until the result of the inoculation was established, the only contact with the outside world during this period being the introduction of food. Any pig inoculated with test material which failed to show symptoms of swine fever—showing that the virus had been destroyed—was given a test dose of live virus in order to prove its susceptibility. A careful *post-mortem* examination of every pig was made and the spleen cultured to exclude bacterial infections, and when it appeared doubtful whether a pig was actually infected with swine fever, a healthy pig was placed in the same sty as a contact, or spleen filtrates were used to inoculate another pig. In the experiments of Uhlenhuth, Miessner and Geiger, there was sometimes a considerable interval between the inoculation of the test material and the first appearance of symptoms. This delayed reaction they attributed to a weakening of the virus by the disinfectant, but in the work here described no delayed reactions occurred. The virus was either completely inactivated or completely unaltered.

Summary and Conclusions.

The viability of the swine-fever virus in acid and alkaline media has been studied. All that can be said is that the virus is very resistant to changes in the hydrogen-ion concentration.

Infected brick and hay were exposed to atmospheric influences. As other workers have found, the virus survives a few days only. In association with these experiments, pigs were exposed to infection by feeding virus, by dropping virus into the eye and by injecting virus into the nasal cavities. Although all these pigs were fully susceptible to swine fever, none of them developed the disease until

they were later inoculated subcutaneously with virus to prove their susceptibility. These results confirm the work of other workers and explain the irregular results of contact experiments.

The resistance of the virus to chemical disinfection was investigated. The method of testing is new and gives consistent results. Using this method, the virus was destroyed in 15 minutes by a 5 per cent. solution of pure phenol and by a solution of hypochlorite containing 1·66 per cent. available chlorine. The hypochlorite is to be preferred because of its cleansing and deodorant action: it has the disadvantage that its action is less persistent than that of phenol.

It is a pleasure to record my appreciation of the assistance given by Dr. Coyne, Dr. Kennedy and other members of the staff of Imperial Chemical Industries, Ltd., in this investigation.

TABLE I

No. of Pig.	*Virus-containing material.*	*pH.*	*Interval between setting of pH and inoculation.*	*Result.*
811	Haemolysed blood	4·0	1 hour	Died S.F. 14 days
803	Haemolysed blood	6·0	1 hour	Died S.F. 18 days
802	Haemolysed blood	8·0	1 hour	Died S.F. 6 days
805	Haemolysed blood	10·8	1 hour	Died S.F. 7 days
804	Haemolysed blood	Control	1 hour	Died S.F. 15 days
824	Serum diluted 1—10	4·0	1 hour	Died S.F. 6 days
820	Serum diluted 1—10	6·0	1 hour	Died S.F. 9 days
822	Serum diluted 1—10	8·0	1 hour	Died S.F. 9 days
825	Serum diluted 1—10	10·0	1 hour	Died S.F. 9 days
843	Serum diluted 1—10 Filtered before storage	4·0	78 days	Died S.F. 7 days
847	Serum diluted 1—10 Filtered before storage	6·0	78 days	Died S.F. 23 days
858	Serum diluted 1—10 Filtered before storage	8·0	78 days	Died S.F. 9 days
857	Serum diluted 1—10 Filtered before storage	10·0	78 days	Slaughtered after 23 days in moribund condition. S.F.
856	Serum diluted 1—10 Filtered before storage	Control	78 days	Slaughtered after 11 days S.F.
53	Serum diluted 1—10	1·4	1 hour	Virus destroyed. Inoculated 21 days later with live virus. Died S.F. 9 days

No. of pig.	*Virus-containing material.*	*pH.*	*Interval between setting of pH and inoculation.*	*Result.*
31	Serum diluted 1—10	3·0	1 hour	Slaughtered after 11 days in moribund condition. S.F.
35	Serum diluted 1—10	5·0	1 hour	Slaughtered after 10 days in moribund condition. S.F.
29	Serum diluted 1—10	9·0	1 hour	Died S.F. 9 days
32	Serum diluted 1—10	11·0	1 hour	Died S.F. 7 days
37	Serum diluted 1—10	13·0	1 hour	Virus destroyed. Inoculation after 21 days with live virus. Slaughtered 9 days later in moribund condition. S.F.

Total number of pigs used :20.

TABLE II

No. of Pig.	*Virus-containing material.*	*Time of Exposure.*	*Result.*
36	Chopped hay	7 days	Slaughtered after 14 days in moribund condition. S.F.
30	Brick	7 days	Slaughtered in moribund condition after 14 days. S.F.
21	Chopped hay	14 days	Virus destroyed. Inoculated 21 days later with live virus. Died S.F. 11 days.
22	Brick	14 days	This pig never fed well after inoculation and was slaughtered after 20 days. Spleen pulp inoculated into another pig did not cause swine fever.
25	Chopped hay	21 days	Virus destroyed. Inoculated with live virus 21 days later. Died S.F. 11 days.
20	Brick	21 days	Virus destroyed. Inoculated with live virus after 21 days. Died S.F. 7 days.
24	Chopped hay	28 days	Virus destroyed. Inoculated with live virus 21 days later. Died S.F. 6 days.
26	Brick	28 days	Virus destroyed. Inoculated with live virus 21 days later. Slaughtered after 18 days in moribund condition. S.F.
827	Brick	21 days Kept in desiccator as control	Died S.F. 15 days.

Total number of pigs used : 10.

TABLE III

No. of Pig.	*Disinfectant.*	*Concentration† Per cent.*	*Virus-containing material.*	*Exposure time.*	*Result.*	*Remarks.*
786	Hypochlorite*	5	5 per cent. spleen suspension	15 mins.	Virus destroyed. Inoculated with live virus after 21 days. Died S.F. 19 days	
788	Hypochlorite	2·5	5 per cent. spleen suspension	15 mins.	Died S.F. 12 days	
17	Hypochlorite	12·5	Spleen-faeces mixture	15 mins.	Virus destroyed. Inoculated with live virus after 21 days. Died S.F. 15 days	
27	Hypochlorite	11·25	Spleen-faeces mixture	15 mins.	Slaughtered after 21 days in moribund state. S.F.	
837	Hypochlorite	5	Brick	15 mins.	Virus destroyed. Inoculated with live virus after 21 days. Died S.F. 11 days	The piece of brick was dropped into 200 c.cm. of disinfectant.
842	Distilled Water	—	Brick	15 mins.	Died S.F. 14 days.	The piece of brick was treated in exactly the same way as for the hypochlorite to prove that washing did not remove the virus.
826	Hypochlorite	2·5	Brick	15 mins.	Died S.F. 10 days	The piece of brick was dropped into 200 c.c.m of disinfectant.
827	Distilled Water	—	Brick	15 mins.	Died S.F. 14 days	The piece of brick was treated in exactly the same way as for the hypochlorite to prove that washing did not remove the virus.

TABLE III—*continued*

No. of Pig.	*Disinfectant.*	*Concentration.† Per cent.*	*Virus-containing material.*	*Exposure time.*	*Result.*	*Remarks.*
15	Hypochlorite + Caustic soda	10 + 1	Spleen-faeces mixture	15 mins.	Died S.F. 8 days	
833	Caustic soda	1	Spleen-faeces mixture	15 mins.	Died S.F. 7 days.	
38	Caustic soda	1	Spleen-faeces mixture	1 hour	Died S.F. 6 days	
828	Caustic soda	2	Spleen-faeces mixture	15 mins.	Died S.F. 24 days	
832	Phenol	3	5 per cent. spleen suspension	1 hour	Slaughtered after 22 days. S.F.	Sufficient 90 per cent. phenol solution was added to the spleen suspension to give concentration shown in Col. 3.
829	Phenol	3	5 per cent. spleen suspension	5½ hours	Pig died suddenly 15 days later. Rupture of auricle. Spleen filtrate injected into another pig, No S.F.	90 per cent. phenol solution as for preceding experiment.
16	Phenol	5	Spleen-faeces mixture	15 mins.	Virus destroyed. Inoculated after 21 days with live virus. Slaughtered 11 days later in moribund condition. S.F.	

TABLE III—*continued*

No. of Pig.	*Disinfectant.*	*Concentration.† Per cent.*	*Virus-containing material.*	*Exposure time.*	*Result.*	*Remarks.*
48	Phenol	2·5	Spleen-faeces mixture	15 mins.	Slaughtered after 14 days in moribund condition. S.F.	
57	Lysol	2	Spleen-faeces mixture	15 mins.	Slaughtered in moribund condition 14 days later. S.F.	Slight symptons of poisoning following intravenous injection.
58	Formalin	1	Spleen-faeces mixture	15 mins.	Died S.F. 7 days	
54	Corrosive sublimate	0·2	Spleen-faeces mixture	15 mins.	Died S.F. 7 days	Slight symptons of poisoning following intravenous injection.
60	Copper sulphate	1	Spleen-faeces mixture	15 mins.	Slaughtered after 14 days in moribund condition. S.F.	

* The undiluted hypochlorite solution contained 11·35 per cent. available Cl and 12·62 per cent. total Cl. (W/W).

† Except when otherwise stated the figures in Column 3 refer to the concentration of disinfectant actually added to the material to be disinfected and not to the final concentration in the mixture.

The following dilutions of the above disinfectants were tested :—

Hypochlorite.—100 per cent., 50 per cent., 25 per cent., 10 per cent., 5 per cent., 1·25 per cent. and 1 per cent. using 5 per cent. spleen suspension alone. Using the spleen-faeces mixture 20 per cent. (twice) 15 per cent. (twice), 12·5 per cent., 11·25 per cent., 10 per cent., 10 per cent. plus 1 per cent. caustic soda, and 5 per cent. were tested.

Caustic Soda.—2 per cent., 1 per cent. (15 mins.) and 1 per cent. (1 hour) using spleen-faeces mixture.

Phenol.—3 per cent. (1 hour, 5½ hours, 24 hours), **6 per cent. (24 hours)**, using 5 per cent. spleen suspension. 2·5 per cent. and 5 per cent. with spleen-faeces mixture.

Total number of pigs used : 52.

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